(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 September 2002 (19.09.2002)

PCT

(10) International Publication Number
WO 02/072616 A2

(51) International Patent Classification[7]: C07K 2/00, 5/09, 5/103, 7/06, C12N 15/88, A61K 38/02, 38/06, 38/07, 38/08, 39/00, 48/00, G01N 33/68

(21) International Application Number: PCT/GB02/01215

(22) International Filing Date: 14 March 2002 (14.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0106315.5 14 March 2001 (14.03.2001) GB

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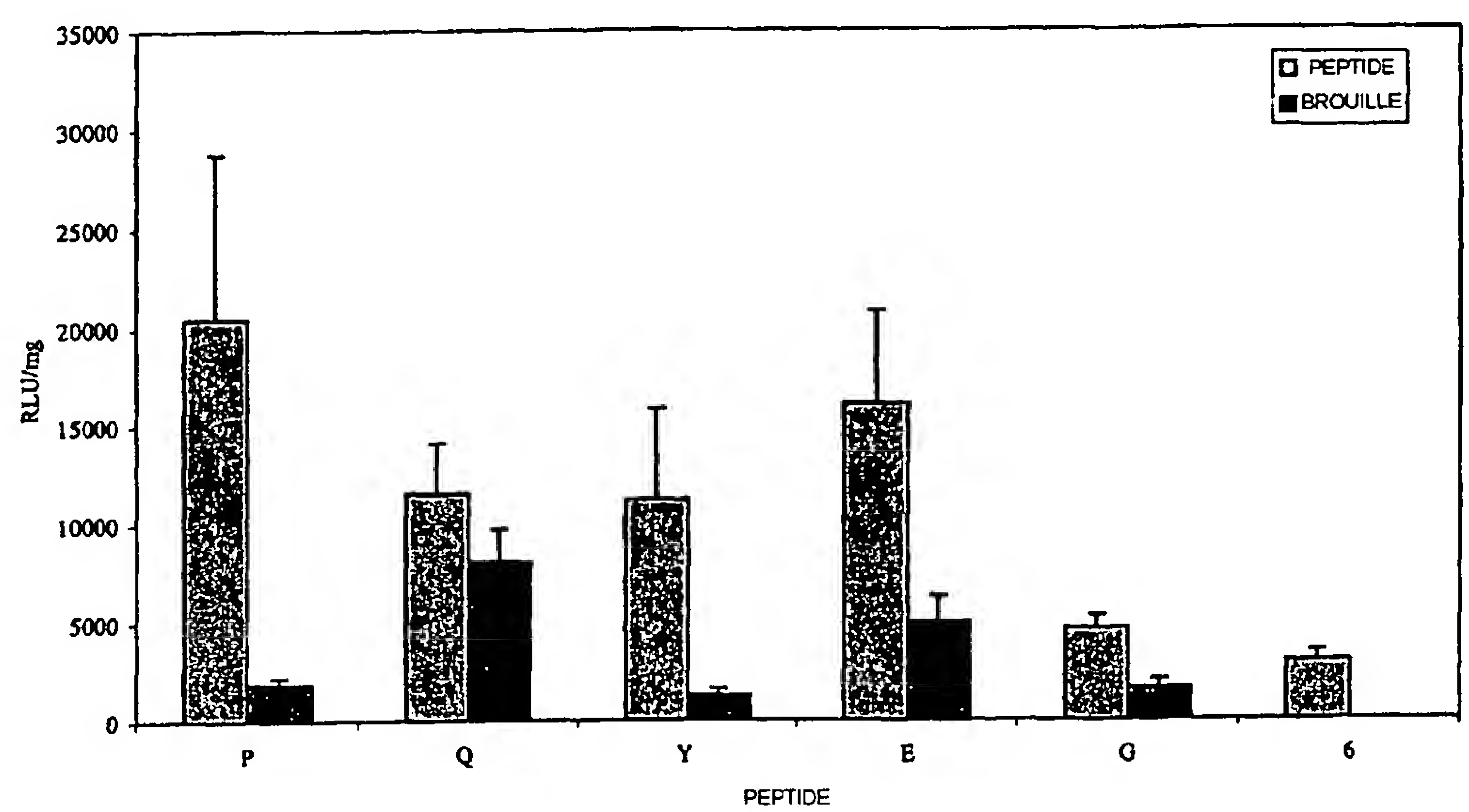
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TRANSFECTION COMPLEXES



(57) Abstract: The invention provides a peptide having at least 3 amino acids comprising an amino acid sequence selected from a) X^1SM [SEQ.ID.NO.:1] b) LX^2HK [SEQ.ID.NO.:2] c) PSGX^3ARA [SEQ.ID.NO.:9] d) SX^4RSMNF [SEQ.ID.NO.:16] e) LX^5HKSMP [SEQ.ID.NO.:18] in which X is a basic amino acid residue, X^1 is Q or P, X^2 is A or T, X^3 is an acidic amino acid residue and X^4 is P or Q. the invention further provides non-viral cell-targeting vector complexes and methods associated therewith.

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TRANSFECTION COMPLEXES

The present invention relates to peptides for use in an improved method of transfecting cells.

The term "transfection" is used herein to denote the introduction of a nucleic acid into a cell. The nucleic acid may be of any origin, and the recipient cell may be prokaryotic or eukaryotic.

Gene therapy and gene vaccination are techniques that offer interesting possibilities for the treatment and/or prophylaxis of a variety of conditions, as does anti-sense therapy. Such techniques require the introduction of a DNA of interest into target cells. The ability to transfer sufficient DNA to specific target cells remains one of the main limitations to the development of gene therapy, anti-sense therapy and gene vaccination. Both viral and non-viral DNA delivery systems have been proposed. In some cases RNA is used instead of DNA.

Receptor-mediated gene delivery is a non-viral method of gene transfer that exploits the physiological cellular process, receptor-mediated endocytosis to internalise DNA. Examples include vectors targeted against insulin receptors, see for example, Rosenkranz et al Experimental Cell Research 199, 323-329 (1992), asialoglycoprotein receptors, see for example, Wu & Wu, Journal of Biological Chemistry 262, 4429-4432 (1987), Chowdhury et al Journal of Biological Chemistry 268, 11265-11271 (1993), and transferrin receptors, see for example, Ciriel et al, Proc. Natl. Acad. Sci. USA 88, 8850-8854 (1991). Further examples of vectors include monoclonal antibodies targeting receptors on neuroblastoma cells (Yano et al, 2000), folate conjugated to liposomes (Reddy & Low 2000, Reddy et al. 1999), galactose for targeting liver cells (Han et al. 1999 Bettinger et al. 1999) and

asialogylcoprotein, also for liver cells (Wu et al. 1991).

Receptor-mediated non-viral vectors have several advantages over viral vectors. In particular, they lack pathogenicity; they allow targeted gene delivery to specific cell types and they are not restricted in the size of nucleic acid molecules that can be packaged. Gene expression is achieved only if the nucleic acid component of the complex is released intact from the endosome to the cytoplasm and then crosses the nuclear membrane to access the nuclear transcription machinery. However, transfection efficiency is generally poor relative to viral vectors owing to endosomal degradation of the nucleic acid component, failure of the nucleic acid to enter the nucleus and the exclusion of aggregates larger than about 150nm from clathrin coated vesicles.

Desirable properties of targeting ligands for vectors are that they should bind to cell-surface receptors with high affinity and specificity and mediate efficient vector internalisation. Short peptides have particular advantages as targeting ligands since they are straightforward to synthesise in high purity and, importantly for *in vivo* use, they have low immunogenic potential.

WO 98/54347 discloses a mixture comprising an integrin-binding component, a polycationic nucleic acid-binding component, and a lipid component, and also discloses a complex comprising
(i) a nucleic acid, especially a nucleic acid encoding a sequence of interest,
(ii) an integrin-binding component,
(iii) a polycationic nucleic acid-binding component, and
(iv) a lipid component.
The complex is primarily an integrin-mediated transfection vector.

Integrins are a super-family of heterodimeric membrane proteins consisting of several different α and ß subunits. They are important for attachment of cells to the extracellular matrix, cell-cell interactions and signal transduction. Integrin-mediated internalisation proceeds by a phagocytic-like process allowing the internalisation of bacterial cells one to two micrometers in diameter (Isberg, 1991). Targeting of non-viral vectors to integrins, therefore, has the potential to transfect cells in a process that mimics infection of cells by pathogens and avoids the size limitation imposed by clathrin-coated vesicles in receptor-mediated endocytosis.

It is considered that the components described in WO 98/54347 associate electrostatically to form the vector complex, the vector being of the lipopolyplex type. The vector complexes of WO 98/54347 are found to transfect a range of cell lines and primary cell cultures with high efficiency, with integrin specificity and with low toxicity. For example, vascular smooth muscle cells are transfected with 50% efficiency, endothelial cells with 30% efficiency and haematopoietic cells with 10% efficiency. Furthermore, *in vivo* transfection of bronchial epithelium of rat lung and pig lung with an efficiency comparable with that of an adenoviral vector has been demonstrated.

Vectors that utilise integrin receptors to mediate gene transfer have the advantage that they target a large number of different types of cells in the body as integrin receptors are relatively widespread. In some circumstances, for example, in *in vivo* treatment, however, it may be preferable to target recipient cells more specifically.

It is an object of the present invention to provide improved vector complexes with enhanced cell targeting properties. The present invention is based on the development of

synthetic targeting non-viral vector complexes that carry a ligand that is more cell-type selective than the ligands of the prior art.

Previous approaches to targeted non-viral vectors have included the use of antibodies to substances involved in cell-cell adhesion. For example, vectors including monoclonal antibodies that target receptors on neuroblastoma cells (Yano et al, 2000) are known. Further examples of targeting systems have proposed galactose for targeting liver cells (Han et al. 1999 Bettinger et al. 1999) and asialogylcoprotein, also for liver cells (Wu et al. 1991). However, such methods have been effective only in limited circumstances. For example, antibodies have broad applicability but they are time-consuming to produce and, by virtue of their size, are not as suitable for *in vivo* administration to an organism as a small molecule ligand. Furthermore, the methods previously described do not allow targeting to a cell type for which a ligand is not yet available.

In the development of effective targeting vectors it is useful for several different target-binding ligands to be available. Effective targeted transfection requires not only good targeting but also effective transfer of the vector DNA to the nucleus of the target cell. Even if a ligand is effective in targeting and binding to a target cell, effective gene transfection does not always occur. The reasons for that are, at present, not clear. Accordingly, there remains a degree of unpredictability regarding whether a ligand that binds effectively to a target cell will also bring about effective transfection. It is therefore desirable to have available a "pool" of ligands for any particular cell surface receptor from which an effective transfection ligand may be selected. Such selection may take place by means of a gene transfer assay using, for example, a

reporter gene, or by any other suitable means.

The invention is based on the identification of specific peptide sequences that bind to human airway epithelial (HAE) cells. The identified families of HAE cell surface receptor binding component peptide motifs mediate specific binding to HAE cells.

The present invention provides peptide having consisting of or comprising an amino acid sequence selected from

a) X^1SM [SEQ.ID.NO.:1];
b) LX^2HK [SEQ.ID.NO.:2];
c) PSGX^3ARA [SEQ.ID.NO.:9];
d) SX^4RSMNF [SEQ.ID.NO.:16]; and
e) LX^5HKSMP [SEQ.ID.NO.:18],

in which X^1 is a basic amino acid residue, X^2 is Q or P, X^3 is A or T, X^4 is an acidic amino acid residue and X^5 is P or Q.

Preferably, the peptide of the invention consists of or comprises an amino acid sequence selected from

a) X^1SM [SEQ.ID.NO.:1];
b) LX^2HK [SEQ.ID.NO.:2]; and
c) PSGAARA [SEQ.ID.NO.:3],

in which X^1 is a basic amino acid residue and X^2 is Q or P.

Preferably X^1 is K or R. Preferably X^2 is P. Preferably X^3 is A. Preferably X^4 is E or Q [SEQ.ID.No.::17]. More preferably X^4 is E. Preferably X^5 is P.

Preferably, a peptide of the invention comprises a sequence selected from LQHKSMP [SEQ.ID.NO.:4], LPHKSMP [SEQ.ID.NO.:5], VKSMVTH [SEQ.ID.NO.:6], SERSMNF [SEQ.ID.NO.:7], VGLPHKF [SEQ.ID.NO.:8], YGLPHKF [SEQ.ID.NO.19], PSGAARA [SEQ.ID.NO.:3], SQRSMNF [SEQ.ID.NO.:36] and PSGTARA

[SEQ.ID.NO.:38]. Most preferably, the peptide comprises a sequence selected from LQHKSMP [SEQ.ID.NO.:4], and LPHKSMP [SEQ.ID.NO.:5].

A peptide of the invention may be up to 20 amino acids in length, or may be longer. A peptide of the invention generally has at least 5 amino acids but may have perhaps fewer. Generally, a peptide of the invention has any number of amino acids from 6 to 20 inclusive. The peptide may have 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids. Generally, it is preferred for a peptide of the invention to have 15 amino acids or fewer. For example, a peptide of the invention may have 12 amino acids or fewer. Preferably a peptide of the invention according to the invention has 10 amino acids or fewer. Generally, it is preferred for a peptide of the invention to have 5 or more amino acids. For example, a peptide of the invention may have 6 or more amino acids. For example a peptide of the invention has 7 amino acids. In the case of a peptide comprising amino acid sequence c) above, the minimum size is 7 amino acids.

Preferably, a peptide of the invention is such that X^1 is K or R or X^2 is Q or P.

A peptide of the invention may comprise a cyclic region. Preferably, the motif of the invention is flanked by two or more cysteine residues that are capable of forming one or more disulphide bond(s). For example, a peptide of the invention may be "peptide P'" (CLPHKSMPC [SEQ.ID.NO.:10]) or "peptide Q'" (CLQHKSMPC [SEQ.ID.NO.:11]).

The peptides of the invention find use in HAE cell targeted non-viral transfection vector complexes. They are also useful in targeted viral tranfection vectors.

The peptide is preferably linked to a polycationic nucleic acid binding component. The polycationic nucleic acid binding component may be any polycationic molecule suitable for binding a nucleic acid.

For example, it may be polyethylenimine. Polyethylenimine (PEI) is a non-toxic, cross linked cationic polymer with gene delivery potential (*Proc. Natl. Acad. Sci.*, 1995, **92**, 7297-7301). For example, the peptide may be linked to the PEI structure via a disulphide bridge using methods known in the art (for example, *Gene Therapy*, 1999, **6**, 138-145). Polyethylenimine is obtainable from Fluka (800kDa) or from Sigma (50kDa) or alternatively pre-diluted for transfection purposes from PolyPlus-tranfection (Illkirch, France). Typically, PEI is most efficient when in a 9 fold excess over DNA (the excess ratio being calculated as PEI nitrogen : DNA phosphate) and at pH 5-8. Such parameters may optimised in a manner familiar to the person skilled in the art.

Another example of a nucleic acid-binding polycationic molecule is an oligopeptide comprising one or more cationic amino acids. Such a oligopeptide may, for example, be an oligo-lysine molecule having from 5 to 25 lysine moieties, preferably having from 10 to 20 lysine moieties, for example 16 lysine moieties, an oligo-histidine molecule, or an oligo-arginine molecule or a combined oligomer comprising any combination of histidine, arginine and lysine residues and having a total of from 5 to 25 residues, preferably having from 10 to 20 residues, for example 16 residues.

The peptide may be attached to the polycationic nucleic acid binding component via a spacer.

A spacer element is generally a peptide, that is to say, it comprises amino acid residues. The amino acids may be naturally occurring or non-naturally occurring. They may

have L- or D-configuration. A spacer may have two or more amino acids. It may, for example, comprise three or more amino acids, for example, four or more, for example, five or more, for example, up to ten amino acids or more. The amino acids may be the same or different, but the use of multiple lysine residues (or other cationic amino acids suitable for use in the polycationic nucleic acid-binding component of a vector complex) should be avoided in the spacer as oligolysine sequences have activity as a polycationic nucleic acid-binding component of a vector complex of the present invention.

The spacer may be, for example, the dipeptide glycine-glycine (GG) or glycine-alanine (GA). Generally it is preferable that the spacer is longer and/or more hydrophobic than the dipeptide spacers GG and GA.

The spacer may be more hydrophobic than the dipeptides GG and GA. For example, amino acids that are more hydrophobic than glycine and alanine may be used. Examples of hydrophobic amino acids are well known and include ϵ-amino hexanoic acid.

A spacer may be either longer or more hydrophobic than the dipeptides GG and GA, or it may be both longer and more hydrophobic. An example of the latter type of spacer is XSXGA, wherein S = serine, G = glycine, A = alanine and X = ϵ-amino hexanoic acid. This spacer is highly hydrophobic.

The invention further provides a peptide derivative of formula A-B-C wherein

A is a polycationic nucleic acid-binding component,
B is a spacer element, and
C is a peptide as described above.

Polycationic nucleic acid-binding component A may be any polycationic nucleic acid-binding component as described

above. Spacer element B may be any of the spacer elements described above.

The invention further provides a non-viral transfection complex comprising:

(i) a nucleic acid,

(ii) a lipid component,

(iii) a polycationic nucleic acid-binding component, and

(iv) a cell surface receptor binding component,

comprising a peptide as described above.

The cell surface receptor binding component may have the features described above in relation to the peptides of the invention.

The cell surface receptor binding component peptides were identified by selection from a peptide library of random 7-mers (peptides having seven amino acid residues) and random 12-mers (peptides having twelve amino acid residues) displayed on filamentous phage particles. Results obtained using the random 7-mer library were better than those using the random 12-mer peptide library. The reasons for the difference in performance of the seven and twelve amino acid library are not known at present. It is possible that the larger amino acid insert in the phage coat protein reduces the viability of the phage and/or that the additional protein synthesis requirement places too great a burden on the E.coli bacteria. Alternatively, or in addition, impurities in or defects of the 12-mer library may have adversely affected the outcome of the experiments with that library. It appears, however, that smaller peptides, for example heptameric peptides are preferred. Accordingly, the peptide of the invention preferably has 4 to 11 amino acids, more preferably 4 to 10 amino acids, for example 7 amino acids.

The 7-mer library used was a C7C library (i.e. random 7-mer

peptides flanked by cysteine residues) obtained from New England Biolabs Inc. The 12-mer library used was also obtained from New England Biolabs Inc.

As indicated above, the HAE cell surface receptor binding peptides of the invention were identified by selection from a phage display library comprising random peptide sequences seven residues in length flanked by cysteine residues to allow cyclisation. Such selection procedures are generally known. According to such procedures, suspensions of phage are incubated with target cells. Unbound phage are then washed away and, subsequently, bound phage are extracted either by washing the remaining cells with a low pH buffer or by lysing the cells. *E. coli* are then infected with released phage and a preparation of first round phage is obtained. The cycle is performed repeatedly, for example three times and, in order to enrich for targeting phage, the stringency conditions may be increased in the later rounds of selection, for example by increasing the number of wash steps, introducing a low pH wash prior to elution and preselecting with wells coated with medium blocker.

Following selection by successive rounds of phage amplification, it has been found that phage with high affinity for HAE cells may be selected further by whole cell ELISA using plated HAE cells. Following incubation of the phage with the HAE cells, the cells are washed and retained phage may then be detected by immunostaining. Cell specificity is assessed by comparing phage binding to target cells with phage binding to the wells on which the cells were plated and with phage binding to NIH 3T3 fibroblast control cells.

Using the whole cell ELISA (Enzyme-Linked ImmunoSorbent Assay) assay described above, high affinity and high specificity binding peptides were identified. The cells to

which high affinity phage were bound were lysed to release the bound phage particles. The phage DNA was isolated and sequenced.

The amino acid sequences of clones obtained from cell lysis eluted C7C phage in a first experiment are shown in Table 1a.

Table 1a

Sequence	Clone frequency	SEQ.ID
LQHKSMP	3	4
LPHKSMP	1	5
YGLPHKF	1	19
SERSMNF	3	7
VKSMVTH	2	6
PSGAARA	2	3

The amino acid sequences of clones obtained from cell lysis eluted C7C phage in a second experiment are shown in Table 1b.

Table 1b

Sequence	Clone Frequency	SEQ.ID.NO.
SERSMNF	18	7
YGLPHKF	12	19
PSGAARA	9	3
LQHKSMP	3	4
VKSMVTH	3	6
SQRSMNF	2	36
QPLRHHQ	2	37
LPHKSMP	1	5
PSGTARA	1	38
KQRPAWL	1	39
IPMNAPW	1	40
SLPFARN	1	41
GPARISF	1	42
MGLPLRF	1	43

The 56 sequenced clones from the third round of panning of HAEo- in the second experiment were represented by the 14 sequences shown in Table 1b, with some sequences being represented by multiple phage clones. The sequences shown were each flanked by two cyteine residues in the phage and are thus constrained in a loop formation by disulphide bonds between them. For the avoidance of doubt, all of the sequences in Tables 1a and 1b form part of the present invention.

An analysis of the motifs found in the positive clone amino acid sequences of Table 1a (the first experiment) is shown in

Table 2a.

Table 2a

Motif	Sequence	SEQ.ID.	Clone frequency	Motif frequency
KSM/RSM	LQHKSMP	4	3	9
	LPHKSMP	5	1	
	VKSMVTH	6	2	
	SERSMNF	7	3	
LXHK	LQHKSMP	4	3	5
	LPHKSMP	5	1	
	YGLPHKF	19	1	
LXHKSMP	LQHKSMP	4	3	4
	LPHKSMP	5	1	
PSGAARA*	PSGAARA	3	2	2

* PSGAARA is not a motif, but a repeated clone in the first experiment not containing any motifs already identified.

An analysis of the motifs found in the positive clone amino acid sequences of Table 1b (the second experiment) is shown in Table 2b.

Table 2b

Motif	Sequence	SEQ.ID.	Clone frequency	Motif frequency
KSM/RSM	SERSMNF	7	18	27
	SQRSMNF	36	2	
	VKSMVTH	6	3	
	LQHKSMP	4	3	
	LPHKSMP	5	1	
SXRSMNF	SERSMNF	7	18	20
	SQRSMNF	36	2	
LXHK	LQHKSMP	4	3	16
	LPHKSMP	5	1	
	YGLPHKF	19	12	
PSGXARA	PSGAARA	3	9	10
	PSGTARA	38	1	
LXHKSMP	LPHKSMP	5	3	4
	LQHKSMP	4	1	

The sequences found in the first experiment (Table 1a) were compared and ranked for their binding strength by ELISA using a range of phage titres (Table 3). In Table 3, the sequences are ranked in order of binding affinity to HAE cells. It was found that the sequence LPHKSMP ("Peptide P") had the highest binding affinity.

Table 3

Sequence	SEQ. ID	Clone frequency	Motifs
LPHKSMP	5	1	LXHK, LXHKSMP, KSM
LQHKSMP	4	3	LXHK, LXHKSMP, KSM
YGLPHKF	19	1	LXHK
VKSMVTH	6	2	KSM
PSGAARA	3	2	PSGAARA
SERSMNF	7	3	RSM

From the Tables it may be seen that the motifs KSM/RSM and LXHK were present in several of the clones. This strongly suggests that those motifs are important for HAE cell surface binding. It is at present not known to which HAE receptor(s) the sequences bind. The various motifs may target the same receptor or they may target different receptors.

Good binding indicates a high affinity interaction and/or the binding of a cell surface receptor molecule present in high numbers on the cell surface. The LPHK version of the LXHK motif provides better binding than the LQHK version and the KSM version of the XSM motif provides better binding than the RSM version. The LXHK motif and the KSM motif are frequently found together. This may be due to a cooperative effect, possibly due to the motifs binding to two cell surface receptor molecules.

Although the peptide sequences of the invention were identified using HAE cells, their utility is not limited to use with HAE cells. The receptors to which the peptides bind may be expressed in other cell types. Cell types with which

peptides of the invention may be used may be identified by any suitable screening procedure.

The transfection properties the vector complexes of the invention were investigated in HAE cell transfection experiments as described below.

Non-viral transfection vector complexes incorporating the identified sequences were prepared. Peptides were synthesised using standard solid phase synthetic chemistry and a sixteen-lysine tail was added. The most frequently occurring peptides were chosen for synthesis, with peptide LPHKSMP chosen because it contains three motifs. Each peptide was assigned a single letter name. The peptides chosen for synthesis are shown in Table 4.

Table 4

Peptide	Sequence	SEQ. ID.	Clone frequency	Motifs
E	SERSMNF	7	18	RSM, SXRSMNF
Y	YGLPHKF	19	12	LXHK
G	PSGAARA	3	9	PSGXARA
V	VKSMVTH	6	3	KSM
Q	LQHKSMP	4	3	LXHK, LXHKSMP, KSM
P	LPHKSMP	5	1	LXHK, LXHKSMP, KSM

(Where X = any amino acid)

Luciferase reporter gene DNA was used as the transfection DNA. Transfection complexes were made by mixing the components in the order 1) lipid, then 2) peptide and, finally 3) DNA, followed by dilution. The vector complex suspension was applied to HAE cells and control cells.

Vector complexes incorporating peptide Q ($[K]_{16}$-GACLQHKSMPCG [SEQ.ID.NO.:12]) and vector complexes incorporating peptide P ($[K]_{16}$-GACLPHKSMPCG [SEQ.ID.NO.:13]) were synthesised and compared with vector complexes incorporating peptide S ($[K]_{16}$-GACYKHPGFLCG] [SEQ.ID.NO.:14]) which is a control peptide having the same amino acid constituents as peptide P but in a randomised order (the "scambled control"), Peptide 12 ($[K]_{16}$-XSXGACRRETAWACG [SEQ.ID.NO.:15]), a targeting peptide known to bind to alpha 5 beta 1 integrins and Peptide K ($[K]_{16}$) a DNA binding moiety with no targeting ligand attached.

Transfections of HAE cells and 3T3 cells were performed in 96 well plates containing 20,000 cells plated 24 hours earlier. In the transfection vector complex, peptide to DNA charge ratios (+/-) were used at 1.5:1, 3:1 and 7:1. At physiological pH, DNA carries negative charge and the polycationic-nucleic acid binding component carries positive charge. The "charge ratio" is accordingly the ratio of the charges of the two components in the complex. The lipid component was maintained at a constant proportion, by weight, relative to DNA of 0.75:1. The results of the transfection experiments are shown in Figure 3.

At a 7:1 charge ratio, the transfection efficiency of vector complexes containing peptide P was five-fold higher than the next best peptide, peptide 12 at a 3:1 charge ratio. Peptide P was 150-fold better than peptide S (the scrambled control) at the charge ratio of 7:1 indicating that the transfection efficiency was receptor specific. Vector complexes containing peptide P were almost nine-fold more efficient those containing peptide K, again indicating receptor specificity. The fact that vector complexes containing peptide K performed better in the assay than vector complexes containing peptide S suggests that steric hindrance by the scrambled motif in peptide S may play a role.

Despite the similar HAE cell surface binding properties of peptide P and peptide Q (See Figure 2), peptide P performed significantly better than peptide Q in the transfection assay. This result suggests that binding properties alone are not sufficient to achieve high efficiency of transfection.

The HAE cell surface receptor binding peptide component for use in the vector complex of the invention may be synthesised using standard solid phase peptide synthesis methods.

The identity of the molecules bound by the peptides used in transfections was explored by carrying out a BLAST search (Tables 5a and 5b). Homologies were found to several molecules of interest which may bind molecules present on the surface of epithelial cells in the lung. Pathogen peptides with homology with the peptides of the invention are shown in Table 5a, whilst cell adhesion molecules with homology with the peptides of the invention are shown in Table 5b.

Table 5a

Peptide	Homology	Protein	Pathogen	Receptor
LPHKSMP/ LQHKSMP	**LHKSM**	Glycoprotein B	Human herpesvirus	Cell surface heparan sulphate
SXRSMNF	**SDRSMN**	Capsid binding protein VP2	Human rhinovirus	ICAM-1 or LDL receptor family
YGLPHKF	**YGLPHK**	Unknown	Legionella pneumophila	Unknown epithelial cell receptors
VKSMVTH	**VKSMITQ**	Adhesin P1	Mycoplasma Pneumoniae	Cell surface sialoglycoproteins

Table 5b

Peptide	Homology	Protein	Species	Receptor
SXRSMNF	**SERSMN**	Selectin	Rat	Cell surface glycoproteins
	ERSMDF	Laminin, alpha 5	Human	Extracellular matrix components including integrins
LXHKSMP	**LPHKNM**	Epithelial caderin (ovumorulin)	Mouse/ rabbit	Dimerises, also binds integrin a-E,b-7

Epithelial cadherin is a molecule which is involved in cell-cell adhesion and forms complexes with β-catenin. Human herpesvirus glycoprotein B binds cell surface heparan sulphate proteoglycans. Selectin binds cell surface glycoproteins. Laminin, alpha 5 is a basement membrane protein found in epithelium. The capsid binding protein VP2 of the rhinovirus binds ICAM-1 or the LDL receptor family of molecules in the upper respiratory tract. P-glycoprotein is a molecular pump molecule which is localised in the cell membrane, and coagulation factor XII has been shown to bind cytokeratins on epithelial cells.

In so far as any motif or any peptide of the invention occurs in a naturally-occurring protein, the peptides of invention do not include such a naturally-occurring full-length protein. Generally, the peptides of the invention are 100 or fewer amino acids in length; preferably the peptides of the invention are 50 or fewer amino acids in length. Typically, they are of sizes described above.

The peptides of the invention finds utility in the study of conditions involving the pathogens and the cell adhesion molecules given in Tables 4a and 4b. They are also useful in

the development of treatments for those conditions.

The nucleic acid component may be obtained from natural sources, or may be produced recombinantly or by chemical synthesis. It may be modified, for example, to comprise a molecule having a specific function, for example, a nuclear targeting molecule. The nucleic acid may be DNA or RNA. DNA may be single stranded or double stranded. The nucleic acid may be suitable for use in gene therapy, in gene vaccination or in anti-sense therapy. The nucleic acid may be or may relate to a gene that is the target for particular gene therapy or may be a molecule that can function as a gene vaccine or as an anti-sense therapeutic agent. The nucleic acid may be or correspond to a complete coding sequence or may be part of a coding sequence.

Alternatively, the nucleic acid may encode a protein that is commercially useful, for example industrially or scientifically useful, for example an enzyme; that is pharmaceutically useful, for example, a protein that can be used therapeutically or prophylactically as a medicament or vaccine; or that is diagnostically useful, for example, an antigen for use in an ELISA. Host cells capable of producing commercially useful proteins are sometimes called "cell factories".

Appropriate transcriptional and translational control elements are generally provided. For gene therapy, the nucleic acid component is generally presented in the form of a nucleic acid insert in a plasmid or vector. In some cases, however, it is not necessary to incorporate the nucleic acid component in a vector in order to achieve expression. For example, gene vaccination and anti-sense therapy can be achieved using a naked nucleic acid.

The nucleic acid is generally DNA but RNA may be used in some

cases, for example, in cancer vaccination. The nucleic acid component may be referred to below as the plasmid component or component "D".

As indicated above, the polycationic nucleic acid-binding component is any polycation that is capable of binding to DNA or RNA. The polycation may have any number of cationic monomers provided the ability to bind to DNA or RNA is retained. For example, from 3 to 100 cationic monomers may be present, for example, from 10 to 20, for example from 14 to 18, especially about 16. An oligolysine is particularly preferred, for example, having from 10 to 20 lysine residues, for example, from 13 to 19, for example, from 14 to 18, for example, from 15 to 17 residues, especially 16 residues i.e. $[K]_{16}$, "K" denoting lysine.

A further preferred cationic polymer is polyethylenimine (*Proc. Natl. Acad. Sci.*, 1995, **92**, 7297-7301).

The polycationic DNA-binding or RNA-binding component may advantageously be linked or otherwise attached to the cell surface receptor-binding component. A combined cell surface receptor-binding component/polycationic DNA-binding or RNA-binding component may be referred to below as component "I". For example, a polycationic DNA-binding or RNA-binding component may be chemically bonded to a cell surface receptor-binding component, for example, by a peptide bond in the case of an oligolysine. The polycationic component may be linked at any position of the cell surface receptor-binding component. Preferred combinations of cell surface receptor-binding component and polycationic DNA-binding or RNA-binding component are an oligolysine, especially $[K]_{16}$, linked via a peptide bond to a peptide, for example, a peptide as described above. A further preferred combination of cell surface receptor-binding component and polycationic DNA-binding or RNA-binding component are a polyethylenimine

linked via a covalent link to a peptide, for example, a peptide as described above. For example such a covalent link may be a disulphide bridge or a succinimidyl bridge.

The lipid component may be or may form a cationic liposome. The lipid component may be or may comprise one or more lipids selected from cationic lipids and lipids having membrane destabilising or fusogenic properties, especially a combination of a cationic lipid and a lipid that has membrane destabilising properties.

A preferred lipid component ("L") is or comprises the neutral lipid dioleyl phosphatidylethanolamine, referred to herein as "DOPE". DOPE has membrane destabilising properties sometimes referred to as "fusogenic" properties (Farhood et al. 1995). Other lipids, for example, neutral lipids, having membrane destabilising properties, especially membrane destabilising properties like those of DOPE may be used instead of or as well as DOPE.

Other phospholipids having at least one long chain alkyl group, for example, di(long alkyl chain)phospholipids may be used. The phospholipid may comprise a phosphatidyl group, for example, a phosphatidylalkanolamine group, for example, a phosphatidyl-ethanolamine group.

A further preferred lipid component is or comprises the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium chloride, referred to herein as "DOTMA". DOTMA has cationic properties. Other cationic lipids may be used in addition to or as an alternative to DOTMA, in particular cationic lipids having similar properties to those of DOTMA. Such lipids are, for example, quaternary ammonium salts substituted by three short chain alkyl groups, and one long chain alkyl group. The short chain alkyl groups may be the same or different, and may be selected from methyl and ethyl

groups. At least one and up to three of the short chain alkyl group may be a methyl group. The long alkyl chain group may have a straight or branched chain, for example, a di(long chain alkyl)alkyl group.

Another preferred lipid component is or comprises the lipid 2,3-dioleyloxy-N-[2-(spermidinecarboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoridoacetate, referred to herein as "DOSPA". Analogous lipids may be used in addition to or as an alternative to DOSPA, in particular lipids having similar properties to those of DOSPA. Such lipids have, for example, different short chain alkyl groups from those in DOSPA.

A preferred lipid component comprises DOPE and one or more other lipid components, for example, as described above. Especially preferred is a lipid component that comprises a mixture of DOPE and DOTMA. Such mixtures form cationic liposomes. An equimolar mixture of DOPE and DOTMA is found to be particularly effective. Such a mixture is known generically as "lipofectin" and is available commercially under the name "Lipofectin". The term "lipofectin" is used herein generically to denote an equimolar mixture of DOPE and DOTMA. Other mixtures of lipids that are cationic liposomes having similar properties to lipofectin may be used. Lipofectin is particularly useful as it is effective in all cell types tested.

A further preferred lipid component comprises a mixture of DOPE and DOSPA. Such mixtures also form cationic liposomes. A mixture of DOPE and DOSPA in a ratio by weight 3:1 DOSPA:DOPE is particularly effective. Such a mixture, in membrane filtered water, is available commercially under the name "Lipofectamine". Mixtures comprising DOPE, DOTMA and DOSPA may be used, for example, mixtures of lipofectin and lipofectamine.

Other cationic lipids are available commercially, for example, DOTAP (Boehringer-Mannheim) and lipids in the Tfx range (Promega). DOTAP is N-[1-(2,3-diolyloxy)propyl]-N,N,N-trimethylammonium methylsulphate. The Tfx reagents are mixtures of a synthetic cationic lipid [N,N,N',N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-2,3-di(oleoyloxy)-1,4-butanediammonium iodide and DOPE. All the reagents contain the same amount of the cationic lipid component but contain different molar amounts of the fusogneic lipid, DOPE.

However, lipofectin and lipofectamine appear to be markedly more effective as the lipid component in LID vector complexes of the present invention than are DOTPA and Tfx agents.

The effectiveness of a putative cell surface receptor-binding component, polycationic DNA-binding or RNA-binding component, or of lipid component or of any combination thereof may be determined readily using the methods described herein.

The efficiency of transfection using a transfection complex as described above as transfection vector is influenced by the ratio lipid component:cell surface receptor-binding component:DNA or RNA. For any chosen combination of components for any particular type of cell to be transfected, the optimal ratios can be determined simply by admixing the components in different ratios and measuring the transfection rate for that cell type, for example, as described herein.

Lipofectin and lipofectamine appear to be particularly effective in enhancing transfection in the system described above. Lipofectin has the advantage that only very small amounts are required. Any side effects that may occur are therefore minimised. A suitable weight ratio between the lipid and the DNA components has been found to be 0.75:1. For any given transfection experiment, this ratio may be optimised using

methods known in the art.

Cells that may be transfected by a transfection vector complex incorporating a peptide of the invention include, for example, endothelial or epithelial cells, for example, cells of the any part of the airway epithelium, including bronchial and lung epithelium, and the corneal endothelium. The airway epithelium is an important target for gene therapy for cystic fibrosis and asthma.

A transfection vector complex as described above may be produced by admixing components (i), (ii), (iii) and (iv).

Although the components may be admixed in any order, it is generally preferable that the lipid component is not added last. In the case where there is a combined cell surface receptor-binding component/polycationic DNA-binding or RNA-binding component it is generally preferable to combine the components in the following order: lipid component; combined cell surface receptor-binding/polycationic DNA-binding or RNA-binding component; DNA or RNA component, for example, in the order: lipofectin, oligolysine-peptide component, DNA or RNA component.

A transfection mixture comprising a cell surface receptor-binding component, a polycationic nucleic acid-binding component, and a lipid component may be used to produce a nucleic acid-containing transfection vector complex as described above by the incorporation of a nucleic acid with the mixture, for example, by admixture. Alternatively, the transfection mixture may be used for the production of a vector complex which comprises, instead of the nucleic acid component, any other component that is capable of binding to the polycationic nucleic-acid binding component, for example, a protein.

The individual components of a transfection mixture of the invention are each as described above in relation to the transfection vector complex. The preferred components, preferred combinations of components, preferred ratios of components and preferred order of mixing, both with regard to the mixture and to the production of a vector complex, are as described above in relation to the transfection vector complex.

A transfection mixture preferably comprises an equimolar mixture of DOPE and DOTMA (lipofectin) as the lipid component and an oligolysine-peptide especially a $[K]_{16}$-peptide as a combined cell surface receptor-binding component/nucleic acid-binding component. The preferred molar ratio lipofectine:oligolysine-peptide is 0.75:4.

The invention further provides a non-viral transfection complex comprising:

(i) a nucleic acid,

(iii) a polycationic nucleic acid-binding component, and

(iv) a cell surface receptor binding component,

comprising a peptide as described above.

The cell surface receptor binding component may have the features described above in relation to the peptides of the invention. The nucleic acid component and the polycationic nucleic acid-binding component may be as described above in relation to the non-viral transfection complex comprising (i), (ii), (iii) and (iv).

The effectiveness of a putative cell surface receptor-binding component and polycationic DNA-binding or RNA-binding component may be determined readily using the methods described herein.

The efficiency of transfection using a transfection complex as described above as transfection vector is influenced by the ratio of cell surface receptor-binding component : polycationic nucleic acid-binding component : DNA or RNA. For any chosen combination of components for any particular type of cell to be transfected, the optimal ratios can be determined simply by admixing the components in different ratios and measuring the transfection rate for that cell type, for example, as described herein.

Cells that may be transfected by a transfection vector complex incorporating a peptide of the invention include, for example, endothelial or epithelial cells, for example, cells of any part of the airway epithelium, including bronchial and lung epithelium, and the corneal endothelium. The airway epithelium is an important target for gene therapy for cystic fibrosis and asthma.

A transfection vector complex as described above may be produced by admixing components (i),(iii) and (iv).

Although the components may be admixed in any order, it is generally preferable to combine the components in the following order: combined cell surface receptor-binding/polycationic DNA-binding or RNA-binding component; DNA or RNA component, for example, in the order: polyethylenimine-peptide component; DNA or RNA component.

A transfection mixture comprising a cell surface receptor-binding component and a polycationic nucleic acid-binding component may be used to produce a nucleic acid-containing transfection vector complex as described above by the incorporation of a nucleic acid with the mixture, for example, by admixture. Alternatively, the transfection mixture may be used for the production of a vector complex which comprises, instead of the nucleic acid component, any other component

that is capable of binding to the polycationic nucleic-acid binding component, for example, a protein.

The individual components of a transfection mixture of the invention are each as described above in relation to the transfection vector complex. The preferred components, preferred combinations of components, preferred ratios of components and preferred order of mixing, both with regard to the mixture and to the production of a vector complex, are as described above in relation to the transfection vector complex.

The present invention also provides a process for expressing a nucleic acid in host cells, which comprises contacting the host cells *in vitro* or *in vivo* with a receptor-targeted vector complex of the invention comprising the nucleic acid and then culturing the host cells under conditions that enable the cells to express the nucleic acid.

The present invention further provides a process for the production of a protein in host cells, which comprises contacting the host cells *in vitro* or *in vivo* with a receptor-targeted vector complex of the invention that comprises a nucleic acid that encodes the protein, allowing the cells to express the protein, and obtaining the protein. The protein may be obtained either from the host cell or from the culture medium.

The present invention further provides a method of transfecting cells comprising subjecting the cells to a vector complex according to the invention.

The invention further provides cells, transfected with a nucleic acid by a method according to the invention, and also the progeny of such cells.

The present invention further provides a disease model for use in testing candidate pharmaceutical agent, which comprises cells transfected by a method according to the invention with a nucleic acid suitable for creating the disease model.

The present invention also provides a pharmaceutical composition which comprises a receptor-targeted vector complex of the invention comprising a nucleic acid in admixture or conjunction with a pharmaceutically suitable carrier. The composition may be a vaccine.

The present invention also provides a method for the treatment or prophylaxis of a condition caused in a human or in a non-human animal by a defect and/or a deficiency in a gene, which comprises administering to the human or to the non-human animal a receptor-targeted vector complex of the invention comprising a nucleic acid suitable for correcting the defect or deficiency.

The present invention also provides a method for therapeutic or prophylactic immunisation of a human or of a non-human animal, which comprises administering to the human or to the non-human animal a receptor-targeted vector complex of the invention comprising an appropriate nucleic acid.

The present invention also provides a method of anti-sense therapy of a human or of a non-human animal, comprising anti-sense DNA administering to the human or to the non-human animal a receptor-targeted vector complex of the invention comprising the anti-sense nucleic acid.

The present invention also provides the use of a receptor-targeted vector complex of the invention comprising a nucleic acid for the manufacture of a medicament for the prophylaxis of a condition caused in a human or in a non-human animal by

a defect and/or a deficiency in a gene, for therapeutic or prophylactic immunisation of a human or of a non-human animal, or for anti-sense therapy of a human or of a non-human animal.

A non-human animal is, for example, a mammal, bird or fish, and is particularly a commercially reared animal.

The nucleic acid, either DNA or RNA, in the vector complex is appropriate for the intended use, for example, for gene therapy, gene vaccination, or anti-sense therapy. The DNA or RNA and hence the vector complex is administered in an amount effective for the intended purpose.

The treatments and uses described above may be carried out by administering the respective vector complex, agent or medicament in an appropriate manner, for example, administration may be topical, for example, in the case of airway epithelia.

In a further embodiment, the present invention provides a kit comprising a receptor-targeted vector complex of the invention comprising a nucleic acid.

The present invention also provides a kit that comprises the following items: (a) a cell surface receptor-binding component; (b) a polycationic nucleic acid-binding component, and (c) a lipid component. Such a kit may further comprise (d) a nucleic acid. Such a nucleic acid may be single-stranded or double stranded and may be a plasmid or an artificial chromosome. The nucleic acid component may be provided by a vector complex suitable for the expression of the nucleic acid, the vector complex being either empty or comprising the nucleic acid. For *in vitro* purposes, the nucleic acid may be a reporter gene. For *in vivo* treatment purposes, the nucleic acid may comprise DNA appropriate for

the correction or supplementation being carried out. Such DNA may be a gene, including any suitable control elements, or it may be a nucleic acid with homologous recombination sequences. It has been found that peptide/DNA/lipid/ polycationic nucleic acid-binding component complexes are especially stable in salt free buffer (for example in water, or 5% dextrose).

The present invention also provides a kit that comprises the following items: (a) a cell surface receptor-binding component; and (b) a polycationic nucleic acid-binding component. Such a kit may further comprise (d) a nucleic acid. Such a nucleic acid may be single-stranded or double stranded and may be a plasmid or an artificial chromosome. The nucleic acid component may be provided by a vector complex suitable for the expression of the nucleic acid, the vector complex being either empty or comprising the nucleic acid. The nucleic acid component may be provided by a vector complex suitable for the expression of the nucleic acid, the vector complex being either empty or comprising the nucleic acid. For *in vitro* purposes, the nucleic acid may be a reporter gene. For *in vivo* treatment purposes, the nucleic acid may comprise DNA appropriate for the correction or supplementation being carried out. Such DNA may be a gene, including any suitable control elements, or it may be a nucleic acid with homologous recombination sequences. It has been found that peptide/DNA/polycationic nucleic acid-binding component complexes are especially stable in salt free buffer (for example in water, or 5% dextrose).

The components (a) to (d) kit are, for example, as described above in relation to a cell surface receptor-targeted transfection vector complex or a mixture as described above.

A kit generally comprises instructions, which preferably indicate the preferred ratios of the components and the

preferred order of use or admixing of the components, for example, as described above. A kit may be used for gene therapy, gene vaccination or anti-sense therapy. Alternatively, it may be used for transfecting a host cell with a nucleic acid encoding a commercially useful protein i.e. to produce a so-called "cell factory".

In a kit of the invention the components including the preferred components are, for example, as described above in relation to a vector complex of the present invention.

The polycationic nucleic acid binding component is preferably an oligolysine, as described above. The lipid component is preferably capable of forming a cationic liposome, and preferably is or comprises DOPE and/or DOTMA, for example, an equimolar mixture thereof, or is or comprises DOSPA, for example, a mixture of DOPE and DOSPA, for example in the weight ratio DOPE:DOSPA of 1:3. The rations between the components are preferably as described above, as is the order of mixing of the components.

Targets for gene therapy are well known and include monogenic disorders, for example, cystic fibrosis, various cancers, and infections, for example, viral infections, for example, with HIV. For example, transfection with the p53 gene offers great potential for cancer treatment. Targets for gene vaccination are also well known, and include vaccination against pathogens for which vaccines derived from natural sources are too dangerous for human use and recombinant vaccines are not always effective, for example, hepatitis B virus, HIV, HCV and herpes simplex virus. Targets for anti-sense therapy are also known. Further targets for gene therapy and anti-sense therapy are being proposed as knowledge of the genetic basis of disease increases, as are further targets for gene vaccination. The present invention enhances the transfection efficiency and hence the

effectiveness of the treatment.

Vector complexes of the invention may be effective for intracellular transport of very large DNA molecules, for example, DNA larger than 125kb, which is particularly difficult using conventional vectors. This enables the introduction of artificial chromosomes into cells.

Transfection of the airways, for example, the bronchial epithelium demonstrates utility for gene therapy of, for example, respiratory diseases, such as cystic fibrosis, emphysema, asthma, pulmonory fibrosis, pulmonary hypertension and lung cancer.

Cystic fibrosis (CF) is the most common monogenic disorder in the Caucasian population. Morbidity is mainly associated with lung disease. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator protein (CFTR), a cell membrane channel that mediates secretion of chloride ions. Correction of this defect in the bronchial cells by CFTR gene transfer will correct the biochemical transport defect and, hence, the lung disease. Clinical trials so far have generated encouraging data but highlighted the need for more efficient, non-toxic vectors.

The enhanced levels of transfection make the method of the invention particularly suitable for the production of host cells capable of producing a desired protein, so-called "cell factories". For long-term production, it is desirable that the introduced nucleic acid is incorporated in the genome of the host cell, or otherwise stably maintained. That can be readily ascertained. As indicated above, the range of proteins produced in this way is large, including enzymes for scientific and industrial use, proteins for use in therapy and prophylaxis, immunogens for use in vaccines and antigens

for use in diagnosis.

Accordingly, the present invention provides a method of testing drugs in a tissue model for a disease, wherein the tissue model comprises transgenic cells obtained by transfecting cells with a nucleic acid by contacting the cell with a receptor-targeted vector complex of the invention comprising a nucleic acid.

The present invention is especially useful with a receptor targeted vector complex that is capable of high efficiency transfection. In a preferred embodiment, the vector complex comprises four modular elements; an oligolysine, especially $[K]_{16}$, DNA-binding or RNA-binding element; a high affinity cell surface receptor-binding peptide, for example, a peptide described herein; a DNA or RNA sequence, optionally in a plasmid, and optionally regulated by a viral promoter and an enhancing element; the cationic liposome DOTMA/DOPE (lipofectin). The combination of oligolysine-peptide/DNA or RNA complex with the cationic liposome formulation DOTMA/DOPE is a potent combination. Alternatively a DOPE/DOSPA formulation may be used instead of or in addition to a DOTMA/DOPE formulation. The optimisation of variables associated with complex formation and the mode of transfection by LID vector complexes has been demonstrated.

The most important variables in the formation of optimal LID transfection complexes appear to be the ratio of the three components and their order of mixing.

The invention further provides a method for identifying a cell surface receptor binding ligand for use in a non-viral transfection vector complex comprising the steps:

a) selecting phage from a phage peptide library according to their binding affinity to cells of interest by bringing the phage into contact with the

cells of interest and washing away non-binding phage and then extracting bound phage particles,

b) repeating step (a) if necessary, and preferably
c) selecting from the phage obtained in steps a) and b) those phage which bind to the cell of interest with high affinity using a whole cell ELISA.

Preferably, the stringency of the wash in step a) is increased after the first round of selection by washing at low pH by washing multiple times.

The following non-limiting Examples illustrate the present invention. The Examples refer to the accompanying drawings, in which:

Figure 1 shows the enhancement of phage binding to HAE cells in successive rounds of selection for the C7C library and the 12mer peptide library starting materials.

Figure 2 shows the binding specificity of individual phage clones in a whole cell ELISA assay. Binding affinity to HAE cells, to 3T3 control cells and to the ELISA plate are shown.

Figure 3 shows the relative efficiency of transfection of HAE cells achieved by transfection complexes according to the invention.

Figure 4 shows the relative efficiency of transfection of HAE cells achieved by transfection complexes according to the invention and scrambled control peptides.

Figure 5 shows the relative efficiency of transfection of Neuro-2A cells achieved by transfection complexes according to the invention and a control peptide, peptide 6.

Figure 6 shows the relative efficiency of transfection of IMR32 cells achieved by transfection complexes according to the invention and a control peptide, peptide 6.

Figure 7 shows the relative efficiency of transfection of rabbit adventitial cells achieved by transfection complexes according to the invention and a control peptide, peptide 6.

Figure 8 shows the relative efficiency of transfection of 3T3 cells achieved by transfection complexes according to the invention and a control peptide, peptide 6.

EXAMPLES

MATERIALS & METHODS

Example 1

Peptide Library

The peptide library used in this study, C7C, was obtained from New England Biolabs Inc. Phage growth, titration and amplification procedures were performed as described in the manufacturer's handbook. The library consisted of random peptide sequences seven residues in length and flanked by cystine residues to allow cyclisation by oxidation in air. The library is likely to contain at least $1x10^9$ different amino acid sequences.

Selection of phage from the library

HAE cells were grown to confluence in 24-well plates. The HAE cells used were 1HAEo- cells obtained as a gift from Dr. Dieter Gruenert of the University of California, San Francisco (now of the University of Vermont). Cells were washed twice in Tris-buffered saline, pH 7.4 (TBS) before bloacking cells with 2 ml 2% Marvel, 5% bovine serum albumin

(BSA)-TBS per well for 30 minutes at 4°C. The blocker was removed and $2x10^{11}$ phage were added in 1ml of 2% Marvel, 5% BAS-TBS. The phage were allowed to bind for 2 hours with shaking at 4°C before washing five tiems with 2% BSA-TBS and 5 minutes shaking at 4°C followed by another five washes with 2% BSA-TBS for a few seconds only. Phage were eluted by the addition of 400 μl 76mM citrate buffer pH 2.5 to the wells for 10 minutes with shaking at 4°C. The eluate was removed and neutralised with 600 μl 1M Tris buffer pH 7.5 and retained as the eluted fraction. The remaining cells were lysed with 1ml 30mM Tris buffer pH 8.0, 1mM EDTA for 1 hour on ice. The cells were scraped from the plate , the eluate transferred to a microcentrifuge tube, and vortexed briefly. That eluate was retained as the cell-associated fraction.

The above described process was repeated three times. In the second and third rounds, the stringency of selection was increased by introduction of preselection steps to remove phage that bind to the plastic or to components in the medium and by increasing the number of washes following phage binding. The number of phage present in each eluate (in plaque forming units, PFU) is shown in Figure 1.

Whole cell HAE cell binding ELISA

Binding of phage to tissue cultured HAE cells was investigated by whole cell ELISA. Approx. 8×10^4 HAE cells in 100 ml Hanks Balanced Salts Solution (HBSS) were added to each well of a 96 well plate and incubated at 37°C until cells had adhered. The cells were washed gently in HBSS before blocking by the addition of 0.5% BSA in HBSS for 30 mins. 1×10^{10} phage particles in blocker solution were added to each well and allowed to bind at room temperature for 40 minutes. Unbound phage were removed by washing twice with HBSS, and bound phage were fixed to the cells by incubation

in 3.7% paraformaldehyde for 10 mins. Cells were washed in PBS and incubated in blocking buffer for 45 mins, followed by three washes in PBS. Bound phage were detected by the addition of horseradish peroxidase (HRP)-conjugated anti-M13 antibody diluted 1:5000 in blocking buffer for 1 hour, before washing three times in PBS and developing the ELISA with 2, 2'-azino-bis(3-ethylbenzthiazoline 6-sulfonic acid) (ABTS) substrate solution and reading the absorbance on a plate-reading spectrophotometer at 405nm. The experiment was repeated using 3T3 cells and using empty wells and the comparison of binding affinities enabled the identification of phage that bound selectively to HAE cells. The results for selected peptides are shown in Figure 2.

Peptide-encoding DNA of 12 phage clones that displayed high HAE cell avidity and specificity were sequenced and the peptide sequence deduced. The sequences deduced are shown in table 1. Three major peptide motifs, KSM/RSM, LXHK and LXHKSMP were identified amongst the 12 sequences and one sequence, PSGAARA that contained none of the other three motifs. The sequences were compared and ranked for their binding strength by ELISA using a range of phage titres (table 3). It was found that the sequence LPHKSMP (peptide P) had the highest binding affinity. This sequence and the closely related peptide LQHKSMP (peptide Q) and a control, scrambled version of peptide P were selected for transfection experiments.

Peptide synthesis

The following oligolysine-peptides were prepared for transfection experiments:

Peptide P: $[K]_{16}$-GACLPHKSMPCG - binds to HAE cells
Peptide Q: $[K]_{16}$-GACLQHKSMPCG - binds to HAE cells
Peptide S: $[K]_{16}$-GACYKHPGFLCG - non-binding control
Peptide 12: $[K]_{16}$-XSXGACRRETAWACG - binds to alpha 5 beta 1

integrins (X=ε-amino hexanoic acid).

K_{16}: DNA binding moiety, no targeting ligand.

The oligolysine-peptides were synthesised using standard solid phase oligopeptide synthesis methods.

Transfection experiments

Peptides identified from phage that displayed desirable cell binding characteristics were synthesised using standard solid-phase peptide synthetic chemistry and a sixteen-lysine tail was attached using standard synthesis methods. Control peptide (S), consisted of the same amino acid constituents as the targeting peptide P but in a randomised order, was synthesised for incorporation into lipopolyplex formulations. Transfections of HAE and 3T3 cells were performed in 96 well plated containing 20,000 cells plated 24 h earlier. In the transfection complex, peptide to DNA charge ratios (+/-) were used at 1.5:1, 3:1 and 7:1. The lipid component was maintained at a constant proportion, by weight, relative to DNA of 0.75:1. Prior to making transfection complexes the lipid component was diluted to a concentration of 15 μg per ml, the peptide was prepared at 0.1 mg/ml and the DNA was at 20 μg per ml. All dilutions were performed with OptiMEM reduced serum tissue culture medium (Life Technologies). Transfection complexes were made by mixing of components in the order 1) lipid then 2) peptide and finally 3) DNA, then diluted with OptiMEM to a concentration relative to the DNA component of 0.25 μg DNA per 200μl which volume was added to each well. Each group was performed in replicates of six. The vector complex suspension was then applied to cells within 5 minutes of preparation. Transfection incubations were performed at 37°C for 4 h. Luciferase reporter gene assays in cell free extracts were performed after 48 h incubation using a kit from Promega according to the

manufacturer's protocol. Light units were standardised to the protein concentration within each extract. The results of the transfection experiments are shown in Figure 3.

At a 7:1 charge ratio the transfection efficiency of complexes containing peptide P was five fold-higher than the next best peptide , peptide 12 at a 3:1 ratio. Peptide P was more than 150-fold better than Peptide S at the charge ratio of 7:1 indicating that the transfection efficiency was receptor specific. Complexes containing peptide P were almost nine-fold higher than K_{16}, again indicating receptor specificity. This result also suggests that peptide S is less than a tenth as good in transfection complexes as peptide K_{16}. This may be explained by steric hindrance by the scrambled motif in peptide S.

The difference in transfection performance between peptides P and Q was unexpected as peptide Q(LQHKSMP) varies from P by a single amino acid residue. This result suggests that binding properties alone are not sufficient to explain the transfection potential of the peptides. These results also suggest that the LID vector complex system may be retargeted to other specific peptides described herein this report and may be useful for targeted gene delivery to epithelial cells *in vivo* or *in vitro*.

Example 2

Example 2 is a similar series of experiment to Example 1, with relatively minor changes in a number of conditions.

<u>Cell lines</u>

The human airway epithelial cell line (HAEo-) was maintained in Eagle's minimal essential medium (MEM) HEPES modification (Sigma, Poole) containing 10% foetal calf serum (FCS),

penicillin and streptomycin, and L-glutamine. The mouse fibroblast cell line 3T3 and the human neuroblastoma cell line IMR32 were grown in Dulbecco's MEM with Glutamax-1, without sodium pyruvate, with 4500mg/L glucose, with pyridoxine (Gibco BRL) with 10% FCS, penicillin and streptomycin added. Neuro-2A cells were maintained in Dulbecco's MEM with Glutamax-1 (Gibco BRL) with 10% FCS, sodium pyruvate, penicillin and streptomycin and non-essential amino acids.

Panning cells in monolayer

HAEo- cells were grown to confluence in 24 well plates. Cells were washed twice in TBS before blocking cells with 2mls 2% Marvel, 5% BSA-TBS per well for 30 mins at 4°C. The blocker was removed and 2 x 10^{11} phage were added in 1ml of 2% Marvel, 5% BSA -TBS. The phage were allowed to bind for 2 hours shaking at 4°C before washing five times with 2% BSA-TBS for 5 mins shaking at 4°C, followed by another five washes with 2% BSA-TBS for a few seconds only. Phage were eluted by the addition of 400□l 76mM citrate buffer pH 2.5 to the wells for 10 mins shaking at 4°C. The eluate was removed and the remaining cells were lysed with 1ml 30mM Tris pH 8.0, 1mM EDTA for 1 hour on ice. The cells were scraped from the plate, the eluate transferred to an eppendorf, and vortexed briefly. This eluate was saved as the cell-associated fraction. The phage from this elution were titrated as plaque forming units (PFU) as described in the literature supplied with the library by NEB, before amplification of the phage in *E.coli ER2738* cells as described in the literature.
For the second round of panning, 2 x 10^{11} of the amplified phage from the previous round was used as the input phage. However, in order to reduce the number of plastic and blocking molecule-binding phage isolated, four pre-selection steps of adding the phage to a blocked well with no cells for 30mins at 4°C was carried out before adding the phage to the HAEo- cells. The stringency of washing as also increased in

both the second and third rounds by the addition of a 10 min wash at 4°C using 1ml 76mM citrate buffer pH3.5. For the third round, 2 x 10^{11} amplified phage from the second round was preselected in 5 blocked wells containing no cells for 30mins each, followed by 1 well for 1 hour at 4°C. Phage binding and elution was as described for the second round. Following titration of the third round eluate, single well isolated plaques were picked, amplified and purified for sequencing and clone binding characterisation by whole cell ELISA.

Phage sequencing

The phage were purified from small scale PEG preps (see suppliers methods) and single stranded phage DNA was prepared for sequencing using the method described in Phage display of Peptides and Proteins Edited by Brian K. Kay, Jill Winter and John McCafferty. Briefly, the protein coat was removed from the sample by phenol chloroform extraction, and the DNA pelleted by ethanol precipitation. Trace salt was washed from the pellet with ice cold 70% ethanol before resuspending the DNA in TE.

Between 50 and 100ng purified DNA was used in a Big Dye terminator cycle sequencing reaction (ABI) using the -96 primer (5'-CCCTCATTAGCGTAACG-3') supplied with the library and purified for loading by ethanol precipitation as described in Big Dye kit instructions. The samples were run on an ABI 377 sequencer and the results analysed using the Vector NTI program.

Whole cell ELISA

Approx. 8 x 10^4 HAE cells in 100ml HBSS were added to each well of a 96 well plate and incubated at 37°C until cells had adhered. The cells were washed gently in HBSS before blocking by the addition of 0.5% BSA in HBSS for 30mins. 1 x 10^{10} phage particles in blocker were added to each well and

allowed to bind at room temperature for 40mins. Unbound phage were removed by washing twice with HBSS, and bound phage were fixed to the cells by incubation in 3.7% paraformaldehyde for 10mins. Cells were washed in PBS and incubated in blocking buffer for 45mins, followed by three washes in PBS. Bound phage were detected by the addition of HRP-conjugated anti-M13 antibody diluted 1:5000 in blocking buffer for 1hour, before washing three times in PBS, developing the ELISA with ABTS solution, and reading the absorbance at 405nm.

Peptide synthesis

The [K]16 - forms of the cyclised peptides (as shown in Table 6) were synthesised by standard solid phase synthesis by Alta Biosciences, Birmingham, and the Department of Chemistry, UCL.

Table 6

Phage peptide	SEQ.ID.	Peptide name	Peptide synthesised	SEQ.ID.
LPHKSMP	5	P	$[K]_{16}$-GACLPHKSMPCG	13
LQHKSMP	4	Q	$[K]_{16}$-GACLQHKSMPCG	12
YGLPHLF	19	Y	$[K]_{16}$-GACYGLPHLFCG	44
SERSMNF	7	E	$[K]_{16}$-GACSERSMNFCG	27
VKSMVTH	6	V	$[K]_{16}$-GACVKSMVTHCG	28
PSGAARA	3	G	$[K]_{16}$-GACPSGAARACG	29
YKHPGFL	21	S/YS	$[K]_{16}$-GACYKHPGFLCG	30
NSFMESR	22	ES	$[K]_{16}$-GACNSFMESRCG	31
AGSARPA	23	GS	$[K]_{16}$-GACAGSARPACG	32
PLSHQMK	24	QS	$[K]_{16}$-GACPLSHQMKCG	33
HPPMSKL	25	PS	$[K]_{16}$-GACHPPMSKLCG	34
RRETEWA	26	6	$[K]_{16}$-GACRRETEWACG	35

For the avoidance of doubt, all of the sequences in Table 5 form part of the present invention.

Transfections

Lipopolyplex formation

Complexes were allowed to form electrostatically in a tube by adding the following components in the following order. 50μl of Lipofectin (Life Technologies Ltd) diluted to a concentration of 30 μg/ml in OptiMEM, followed by 70μl peptide (at varying concentrations in OptiMEM for optimisation of the peptide:DNA charge ratio in the complex), with 50μl of the luciferase reporter plasmid pCILuc at a concentration of 40ug/ml in Optimem added finally. The complex was mixed by pipetting briefly before diluting in Optimem to a final volume of 1.57mls.

Transfection

The media was removed from subconfluent HAEo- cells plated at 2×10^4 cells /well overnight in 96 well plates and 200μl of complex (approx. 0.25μg of plasmid DNA) added to each well, leaving minimal time between preparing the complex and adding to the cells. All transfections were carried out in 6 wells each. The cells were incubated with the complexes for 4 hours before replacing with normal media for 48 hours, after which reporter gene expression was analysed by luciferase assay (Promega).

Luciferase Assay

The cells were rinsed twice with PBS before the addition of 100μl of reporter lysis buffer (Promega, diluted 1 in 5 in dH_2O) to the cells for 20 mins at 4°C before freeze-thawing. 20μl of the lysate was transferred to a white plate and the luciferase was measured by a Lucy1 luminometer following the

addition of 100μl of reagent.

The protein present in each transfection well was calculated using the Bio-Rad protein assay reagent (based on the Bradford assay), adding 20μl from the luciferase test to 200μl of the reagent diluted 1 in 5, incubating for 10 mins at room temperature and reading the absorbance at 590nm. The total protein present per well was calculated from comparison with a range of BSA standards.

The results of the transfection experiments are shown in Figure 4. Transfection of HAEo- cells with phage derived peptides and their scrambled controls was carried out with a range of peptide:DNA charge ratios including 1.5:1, 3:1 and 7:1. The ratio giving the highest transfection efficiency (determined as RLU/mg) for each peptide is shown in the figure. Controls include cells with no transfection complexes added (OptiMEM only) and peptide 6, an integrin binding peptide. Each result is the mean of 6 values and error bars represent the standard deviation about the mean.

Example 3

The transfection experiments described above were repeated using Neuro-2A cells, IMR32 cells, rabbit adventitial fibroblast cells and 3T3 cells. For analysis of transfections of those cell lines, cells were plated to subconfluence overnight before transfecting in the same manner as above, analysing reporter gene expression after 24 hours. The results are shown in Figures 5 to 8.

Transfection of Neuro-2A cells with phage-derived peptides was carried out with a range of peptide:DNA charge ratios

including 1.5:1, 3:1 and 7:1. The ratio giving the highest transfection efficiency (determined as RLU/mg) for each peptide is shown in Figure 5. Controls included cells with no transfection complexes added (OptiMEM only) peptide 6, an integrin binding peptide, and peptide S, the scrambled version of peptide Y. Each result is the mean of 6 values and error bars represent the standard deviation about the mean.

Transfection of IMR32 cells with phage-derived peptides was carried out with a range of peptide:DNA charge ratios including 1.5:1, 3:1 and 7:1. The ratio giving the highest transfection efficiency (determined as RLU/mg) for each peptide is shown in Figure 6. Controls include cells with no transfection complexes added (OptiMEM only) peptide 6, an integrin binding peptide, and peptide S, the scrambled version of peptide Y. Each result is the mean of 6 values and error bars represent the standard deviation about the mean.

Transfection of rabbit adventitial fibroblast cells with phage-derived peptides was carried out with a range of peptide:DNA charge ratios including 1.5:1, 3:1 and 7:1. The ratio giving the highest transfection efficiency (determined as RLU/mg)for each peptide is shown Figure 7. Controls include cells with no transfection complexes added (OptiMEM only) peptide 6, an integrin binding peptide, and peptide S, the scrambled version of peptide Y. Each result is the mean of 6 values and error bars represent the standard deviation about the mean.

Transfection of 3T3 cells with phage-derived peptides was carried out with a range of peptide:DNA charge ratios including 1.5:1, 3:1 and 7:1. The ratio giving the highest transfection efficiency (determined as RLU/mg) for each peptide is shown in Figure 8. Controls include cells with no transfection complexes added (OptiMEM only) peptide 6, an integrin binding peptide, and peptide S, the scrambled

version of peptide Y. Each result is the mean of 6 values and error bars represent the standard deviation about the mean.

It is seen in Figures 5, 6 and 7 that transfection of Neuro-2A cells, IMR32 cells, rabbit adventitial fibroblast cells with the peptides was of similar efficiency or lower than transfection with peptide 6. Only in 3T3 cells (Fig. 8) was the transfection efficiency above that seen with peptide 6, with peptides Q and E showing efficiencies of approximately 1.5 times that seen with peptide 6. All transfections showed efficiencies above that of the scrambled peptide. These results may suggest that the molecules bound by the peptide are present on other cell types and in other species but maybe in altered forms or at different densities compared to HAEo- cells.

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CLAIMS:

1. A peptide having consisting of or comprising an amino acid sequence selected from

a) X^1SM [SEQ.ID.NO.:1];
b) LX^2HK [SEQ.ID.NO.:2];
c) PSGX^3ARA [SEQ.ID.NO.:9];
d) SX^4RSMNF [SEQ.ID.NO.:16]; and
e) LX^5HKSMP [SEQ.ID.NO.:18],

in which X^1 is a basic amino acid residue, X^2 is Q or P, X^3 is A or T, X^4 is an acidic amino acid residue and X^5 is P or Q.

2. A peptide as claimed in claim 1 consisting of or comprising an amino acid sequence selected from

a) X^1SM [SEQ.ID.NO.:1];
b) LX^2HK [SEQ.ID.NO.:2]; and
c) PSGAARA [SEQ.ID.NO.:3],

in which X^1 is a basic amino acid residue and X^2 is Q or P.

3. A peptide as claimed in claim 1 wherein X^1 is K or R.

4. A peptide as claimed in claim 1 wherein X^2 is P.

5. A peptide as claimed in claim 1 wherein X^3 is A.

6. A peptide as claimed in claim 1 wherein X^4 is E or Q.

7. A peptide as claimed in claim 6 wherein X^4 is E.

8. A peptide as claimed in claim 1 wherein X^5 is P.

9. A peptide as claimed in any one of claims 1 to 3 wherein the peptide comprises the sequence LQHKSMP [SEQ.ID.NO.4].

10. A peptide as claimed in any one of claims 1 to 4 or 8

wherein the peptide comprises the sequence LPHKSMP [SEQ.ID.NO.5].

11. A peptide as claimed in any one of claims 1, 2 or 3 wherein the peptide comprises the sequence VKSMVTH [SEQ.ID.NO.6].

12. A peptide as claimed in any one of claims 1, 2, 3, 6 or 7 wherein the peptide comprises the sequence SERSMNF [SEQ.ID.NO.7].

13. A peptide as claimed in any one of claims 1, 2 or 4 wherein the peptide comprises the sequence VGLPHKF [SEQ.ID.NO.8].

14. A peptide as claimed in any one of claims 1, 2 or 4 wherein the peptide comprises the sequence YGLPHKF [SEQ.ID.NO.19].

15. A peptide as claimed in any one of claims 1, 2 or 5 wherein the peptide comprises the sequence PSGAARA [SEQ.ID.NO.3].

16. A peptide as claimed in claim 1 wherein the peptide comprises the sequence SQRSMNF [SEQ.ID.NO.:36].

17. A peptide as claimed in claim 1 wherein the peptide comprises the sequence PSGTARA [SEQ.ID.NO.:38].

18. A peptide as claimed in any one of claims 1 to 17 having 5 to 20 amino acids.

19. A peptide as claimed in any one of claims 1 to 17 having 6 to 12 amino acids.

20. A peptide as claimed in any one of claims 1 to 17 having 7 amino acids.

21. A peptide as claimed in any one of claims 1 to 20 comprising a cyclic region of amino acids.

22. A peptide as claimed in claim 21 wherein the peptide comprises two or more cysteine residues capable of forming one or more disulphide bond(s).

23. A peptide as claimed in any one of claims 1 to 22 wherein the peptide is linked to a polycationic nucleic acid-binding component.

24. A peptide as claimed in claim 23 wherein the polycationic nucleic acid-binding component is polyethylenimine.

25. A peptide as claimed in claim 24 wherein the peptide is linked to the polyethylenimine via a disulphide bond.

26. A peptide as claimed in claim 23 wherein the polycationic nucleic acid-binding component is an oligo-lysine molecule having from 5 to 25 lysine moieties.

27. A peptide as claimed in any one of claims 23 to 26 wherein the peptide is linked to the polycationic nucleic acid-binding component via a spacer element.

28. A peptide as claimed in claim 27 wherein the spacer element is GG or GA or is longer and/or more hydrophobic than the dipeptide spacers GG (glycine-glycine) and GA (glycine-alanine).

29. A peptide as claimed in claim 27 or 28 wherein the spacer element is of formula GA.

30. A peptide derivative of formula A-B-C wherein
A is a polycationic nucleic acid-binding component,
B is a spacer element, and

C is a peptide as claimed in any one of claims 1 to 22.

31. A non-viral transfection complex that comprises
(i) a nucleic acid,
(ii) a lipid component,
(iii) a polycationic nucleic acid-binding component, and
(iv) a cell surface receptor binding component,
comprising a peptide as claimed in any one of claims 1 to 30.

32. A complex as claimed in claim 31, wherein the nucleic acid component is or relates to a gene that is the target for gene therapy, gene vaccination or anti-sense therapy.

33. A complex as claimed in claim 31 or 32, wherein transcriptional and/or translational control elements for the nucleic acid are provided and the nucleic acid is optionally packed in a phage or vector.

34. A complex as claimed in any one of claims 31 to 33, wherein the nucleic acid component is DNA.

35. A complex as claimed in any one of claims 31 to 34, wherein the nucleic acid component is RNA.

36. A complex as claimed in any one of claims 31 to 35, wherein the nucleic acid-binding component has from 3 to 100 cationic monomers.

37. A complex as claimed in any one of claims 31 to 36, wherein the polycationic nucleic acid-binding component is an oligolysine.

38. A complex as claimed in claim 37, wherein the oligolysine has from 10 to 20, especially 16 lysine residues.

39. A complex as claimed in any one of claims 31 to 36,

wherein the polycationic nucleic acid-binding component is polyethylenimine.

40. A complex as claimed in any one of claims 31 to 39, wherein the lipid component is or is capable of forming a cationic liposome.

41. A complex as claimed in any one of claims 31 to 40, wherein the lipid component is or comprises one or more lipids selected from cationic lipids and lipids having membrane destabilising or fusogenic properties.

42. A complex as claimed in claim 41, wherein the lipid component is or comprises the neutral lipid dioleyl phosphatidylethanolamine (DOPE) or a lipid having similar membrane destabilising or fusogenic properties.

43. A complex as claimed in claim 41 or claim 42, wherein the lipid component is or comprises the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) or a lipid having similar cationic properties.

44. A complex as claimed in claim 43, wherein the lipid component is or comprises a mixture of DOPE and DOTMA, especially an equimolar mixture thereof.

45. A complex as claimed in claim 44, which comprises an equimolar mixture of DOPE and DOTMA as the lipid component, a peptide as claimed in any one of claims 1 to 14 as the cell surface receptor-binding component, and $[K]_{16}$ as the polycationic nucleic acid-binding component.

46. A complex as claimed in claim 44 or claim 45, wherein the ratio lipid component:the cell surface receptor-binding component/polycationic nucleic acid-binding component: nucleic acid is 0.75:4:1 by weight or 0.5 nmol:1.25 nmol:0.25 nmol on a molar basis.

47. A complex as claimed in any one of claims 41 to 44, wherein the lipid component is or comprises 2,3-dioleyloxy-N-[2-(spermidinecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium-trifluoridoacetate (DOSPA) or a lipid having similar properties to those of DOSPA.

48. A complex as claimed in claim 47, wherein the lipid component is or comprises a mixture of DOPE and DOSPA, especially a mixture of one part by weight DOPE to 3 parts by weight DOSPA.

49. A complex as claimed in claim 48, which comprises a mixture of DOPE and DOSPA as the lipid component, a peptide as claimed in any one of claims 1 to 14 as the cell surface receptor-binding component, and $[K]_{16}$ as the polycationic nucleic acid-binding component.

50. A complex as claimed in claim 49, wherein the ratio lipid component:polycationic nucleic acid-binding component: nucleic acid is 12:4:1 by weight.

51. A process for the production of a complex as claimed in any one of claims 31 to 50, which comprises admixing components (i), (ii), (iii) and (iv).

52. A process as claimed in claim 51, wherein the components are admixed in the following order: lipid component, cell surface receptor-binding component/polycationic nucleic acid-binding component, nucleic acid.

53. A complex as claimed in any one of claims 31 to 50, obtainable by a process as claimed in claim 51 or claim 52.

54. A non-viral transfection complex that comprises
(i) a nucleic acid,
(iii) a polycationic nucleic acid-binding component, and
(iv) a cell surface receptor binding component,

comprising a peptide as claimed in any one of claims 1 to 30.

55. A complex as claimed in claim 54, wherein the nucleic acid component is as described in any one of claims 32 to 35.

56. A complex as claimed in claim 54 or 55, wherein the polycationic nucleic acid-binding component is polyethylenimine.

57. A complex as claimed in any one of claims 54 to 56, wherein the lipid component is or is capable of forming a cationic liposome.

58. A process for the production of a complex as claimed in any one of claims 54 to 57, which comprises admixing components (i), (iii) and (iv).

59. A process as claimed in claim 58, wherein the components are admixed in the following order: cell surface receptor-binding component/polycationic nucleic acid-binding component, nucleic acid.

60. A complex as claimed in any one of claims 54 to 57 obtainable by a process as claimed in claim 58 or claim 59.

61. A mixture comprising a cell surface receptor-binding component, a polycationic nucleic acid-binding component, and a lipid component, the cell surface receptor-binding component being a peptide as defined in claim 1.

62. A mixture as claimed in claim 61 wherein the cell surface receptor-binding component is a peptide as defined in any one of claims 2 to 22.

63. A mixture as claimed in claim 61 or claim 62, wherein the polycationic nucleic acid-binding component is as defined in

any one of claims 36 to 39.

64. A mixture as claimed in any one of claims 61 to 63, wherein the lipid component is as defined in any one of claims 40 to 44, 47 and 48.

65. A mixture as claimed in claim 44 which comprises an equimolar mixture of DOPE and DOTMA as the lipid component, a peptide as claimed in any one of claims 1 to 22 as the cell surface receptor-binding component, and $[K]_{16}$ as the polycationic component nucleic acid-binding component.

66. A mixture as claimed in claim 65, wherein the ratio lipid component:combined cell surface receptor-binding/polycationic nucleic acid-binding component is 0.75:4 by weight.

67. A mixture comprising a cell surface receptor-binding component and a polycationic nucleic acid-binding component, the cell surface receptor-binding component being a peptide as defined in claim 1.

68. A mixture as claimed in claim 67 wherein the cell surface receptor-binding component is a peptide as defined in any one of claims 2 to 22.

69. A mixture as claimed in claim 67 or claim 68, wherein the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 39.

70. A process for producing a complex as claimed in claim 31, which comprises incorporating a nucleic acid with a mixture as claimed in any one of claims 61 to 66.

71. A process for producing a complex as claimed in claim 54, which comprises incorporating a nucleic acid with a mixture as claimed in any one of claims 67 to 69.

72. A method of transfecting a cell with a nucleic acid, which comprises contacting the cell in vitro or in vivo with a complex as claimed in any one of claims 31 to 50, 53 to 57 or claim 60.

73. A pharmaceutical composition which comprises a complex as claimed in any one of claims 31 to 50, 53 to 57 or claim 60, in admixture or conjunction with a pharmaceutically suitable carrier.

74. A method for the treatment or prophylaxis of a condition caused in human or in a non-human animal by a defect and/or a deficiency in a gene, which comprises administering a complex as claimed in any one of claims 31 to 50, 53 to 57 or claim 60 to the human or to the non-human animal.

75. A method for therapeutic or prophylactic immunisation of a human or of a non-human animal, which comprises administering a complex as claimed in any one of claims 31 to 50, 53 to 57 or claim 60 to the human or to the non-human animal.

76. A method of anti-sense therapy, which comprises administering a complex as claimed in any one of claims 31 to 50, 53 to 57 or claim 60 to a human or to a non-human animal.

77. A complex as claimed in any one of claims 31 to 50, 53 to 57 or claim 60 for use as a medicament or a vaccine.

78. Use of a complex as claimed in any one of claims 31 to 50, 53 to 57 or claim 60 for the manufacture of a medicament for the prophylaxis of a condition caused in a human or a non-human animal by a defect and/or a deficiency in a gene, or for therapeutic or prophylactic immunisation, or for anti-sense therapy.

79. A kit that comprises

(i) nucleic acid,

(ii) a lipid component,
(iii) a polycationic nucleic acid-binding component, and
(iv) a cell surface receptor binding component,
comprising a peptide as claimed in any one of claims 1 to 30.

80. A kit that comprises
(i) nucleic acid,
(iii) a polycationic nucleic acid-binding component, and
(iv) a cell surface receptor binding component,
comprising a peptide as claimed in any one of claims 1 to 30.

81. A method for identifying a cell surface receptor binding ligand for use in a non-viral transfection vector complex comprising the steps:
a) selecting phage from a phage peptide library according to their binding affinity to cells of interest by bringing the phage into contact with the cells of interest and washing away non-binding phage and then extracting bound phage particles,
b) repeating step a) if necessary
c) selecting from the phage obtained in steps a) and b) phage which bind to the cell of interest with high affinity using a whole cell ELISA.

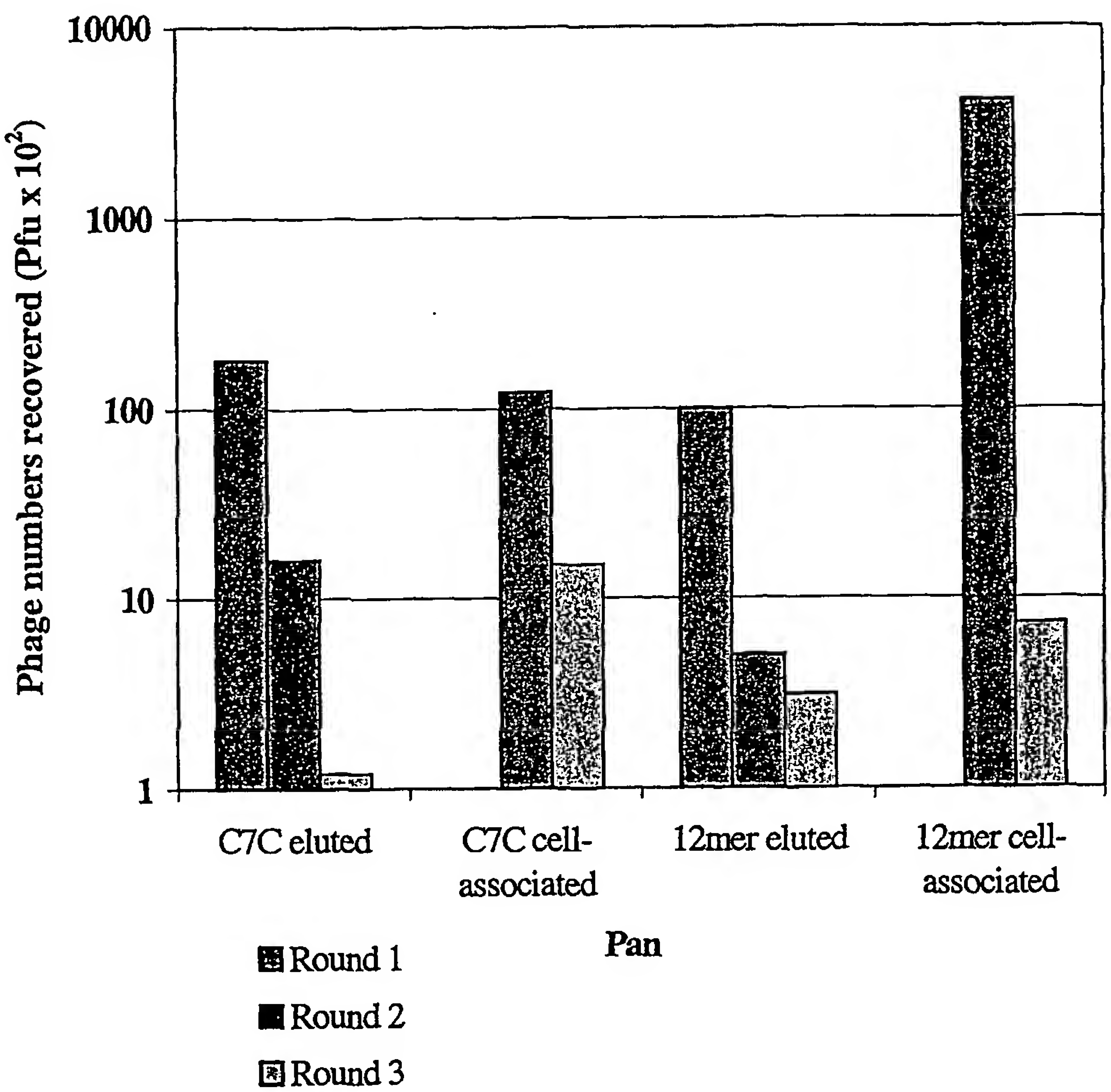
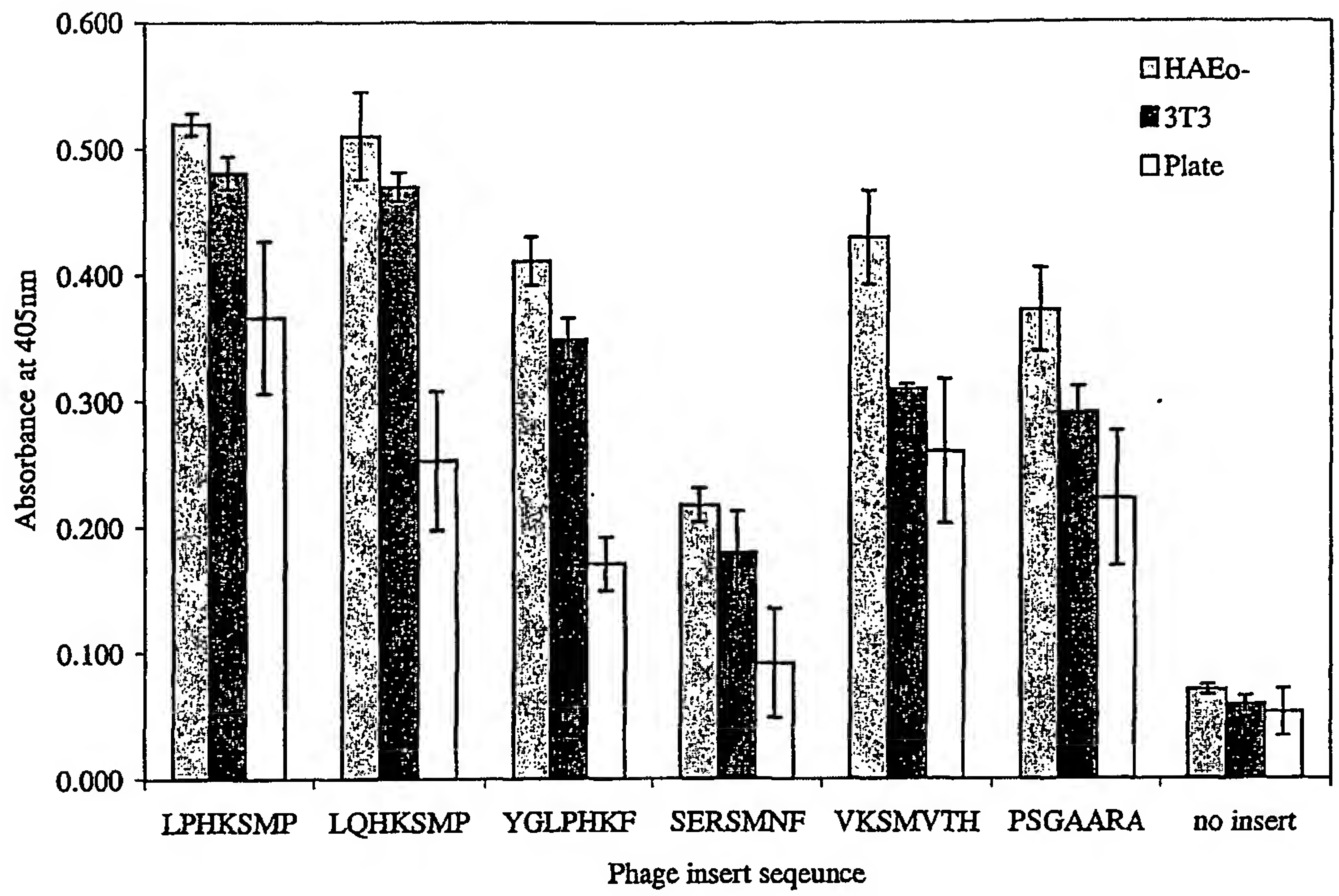

10000
1000
100
10
1
Phage numbers recovered (Pfu x 10^2)
C7C eluted
C7C cell-associated
12mer eluted
12mer cell-associated
Pan
Round 1
Round 2
Round 3

Figure 1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0.200
0.100
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LQHKSMP
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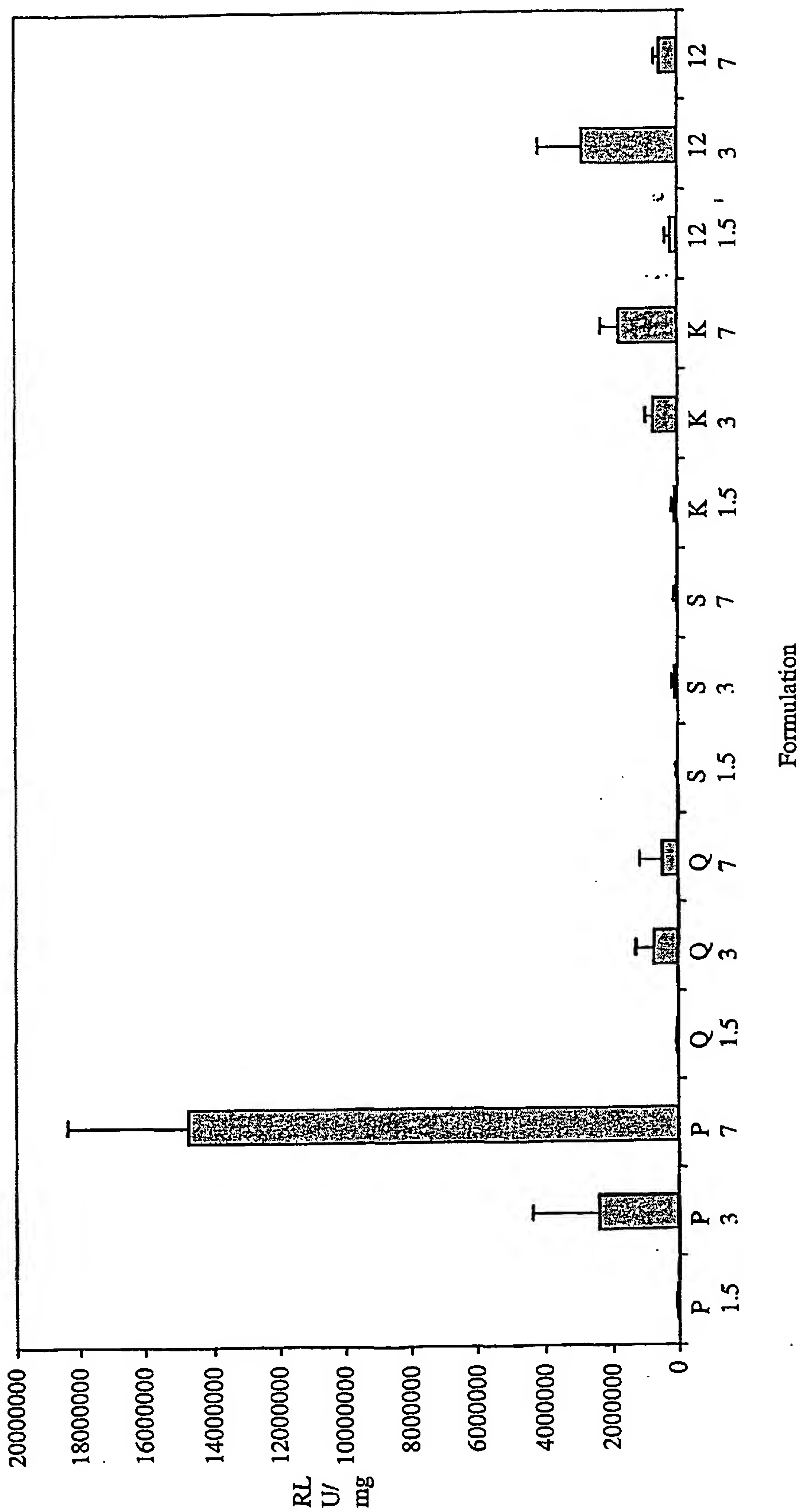
VKSMVTH
PSGAARA
no insert
Phage insert seqeunce

Figure 2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16000000
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P 7
Q 1.5
Q 3
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S 7
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K 3
K 7
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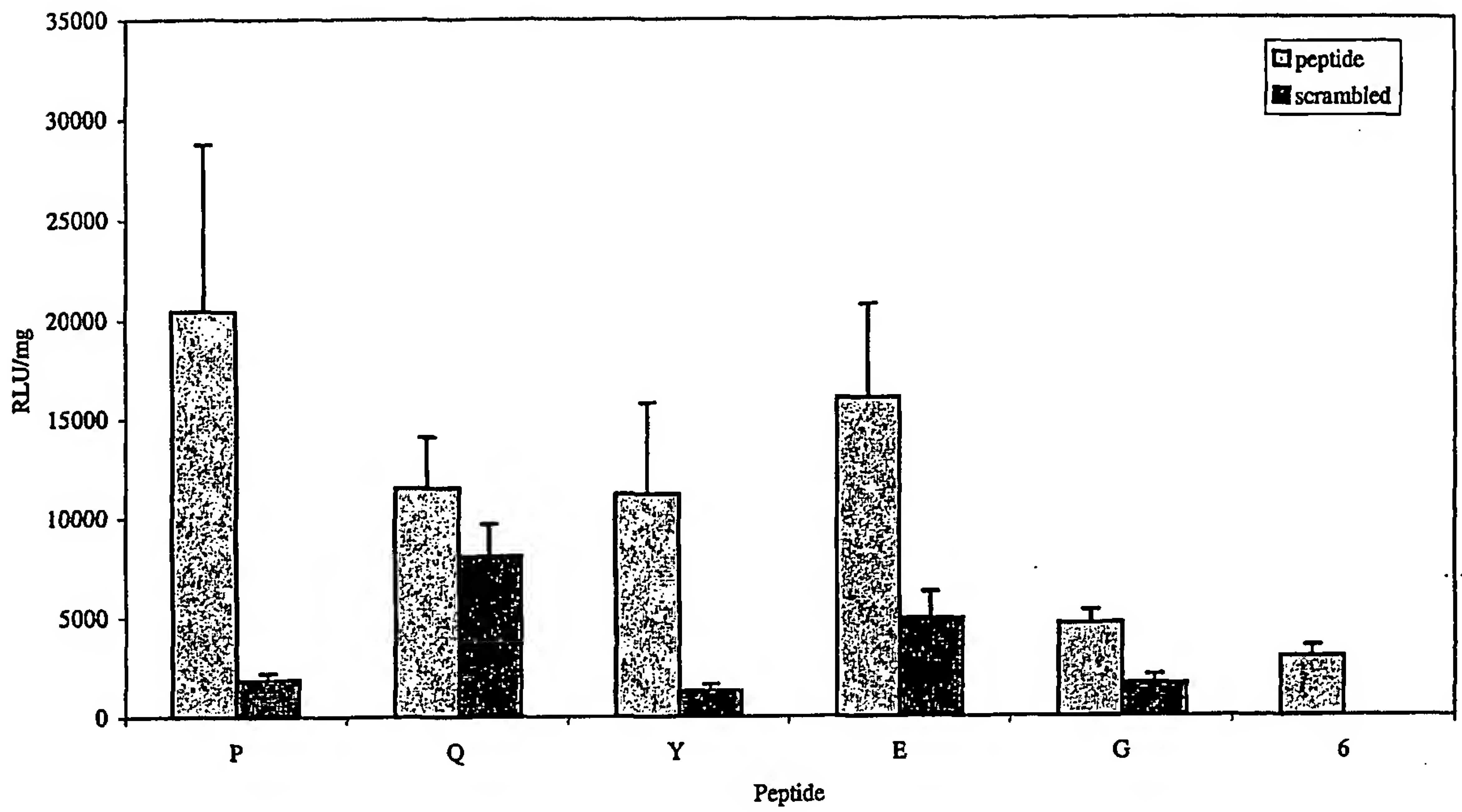
12 7
Formulation

Figure 3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25000
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Q
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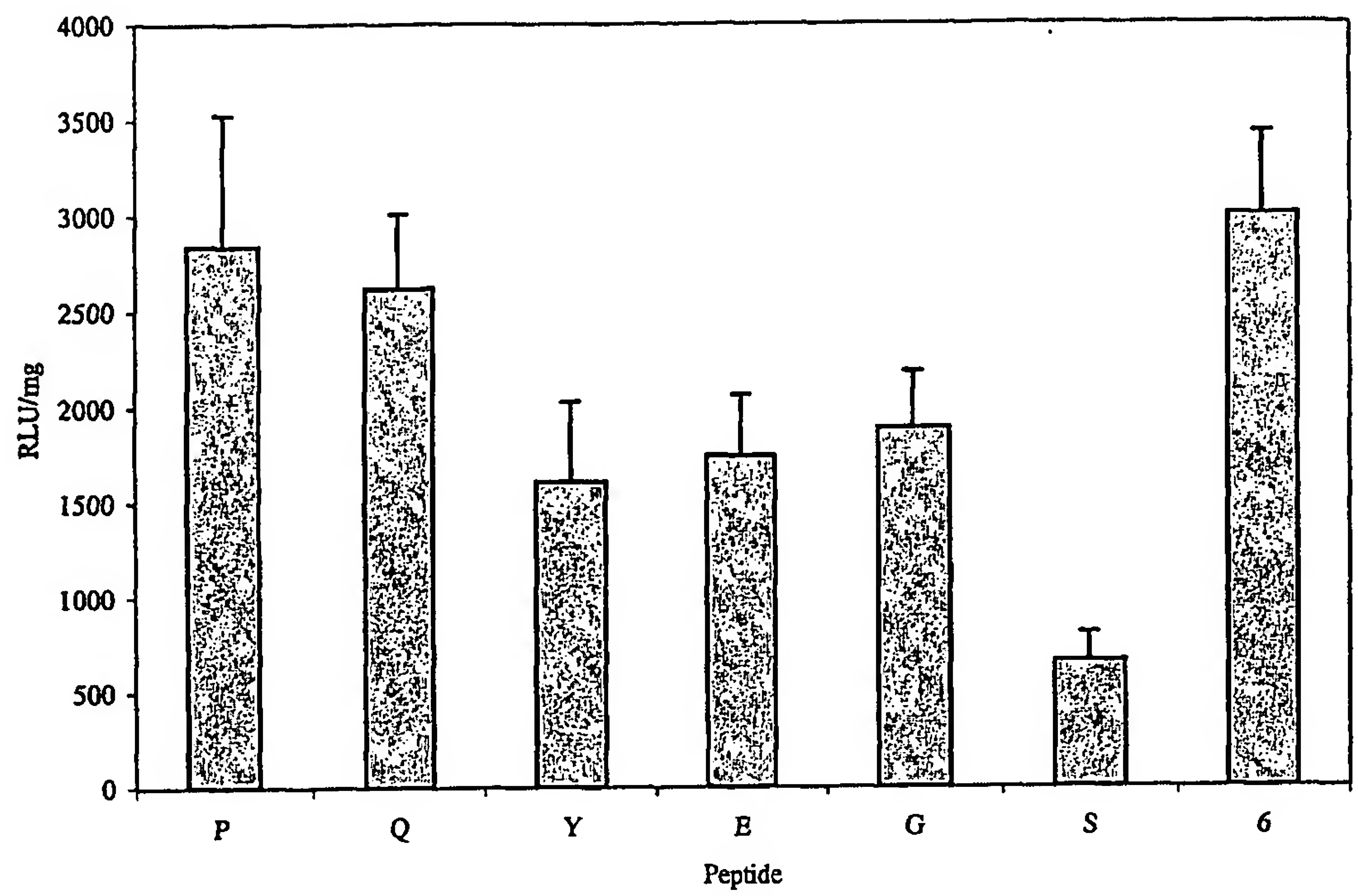
E
G
6
Peptide

Figure 4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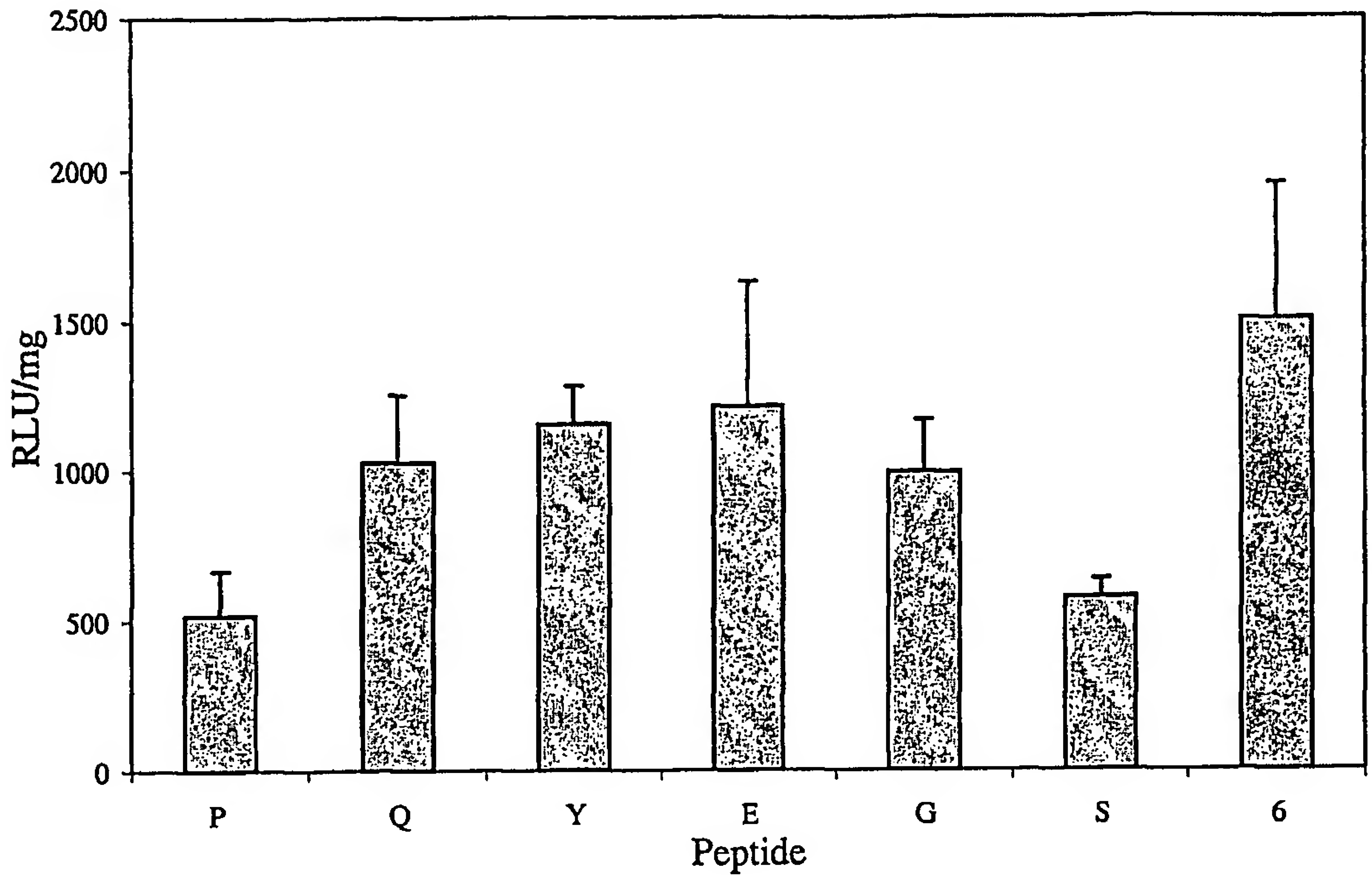
G
S
6
Peptide

Figure 5



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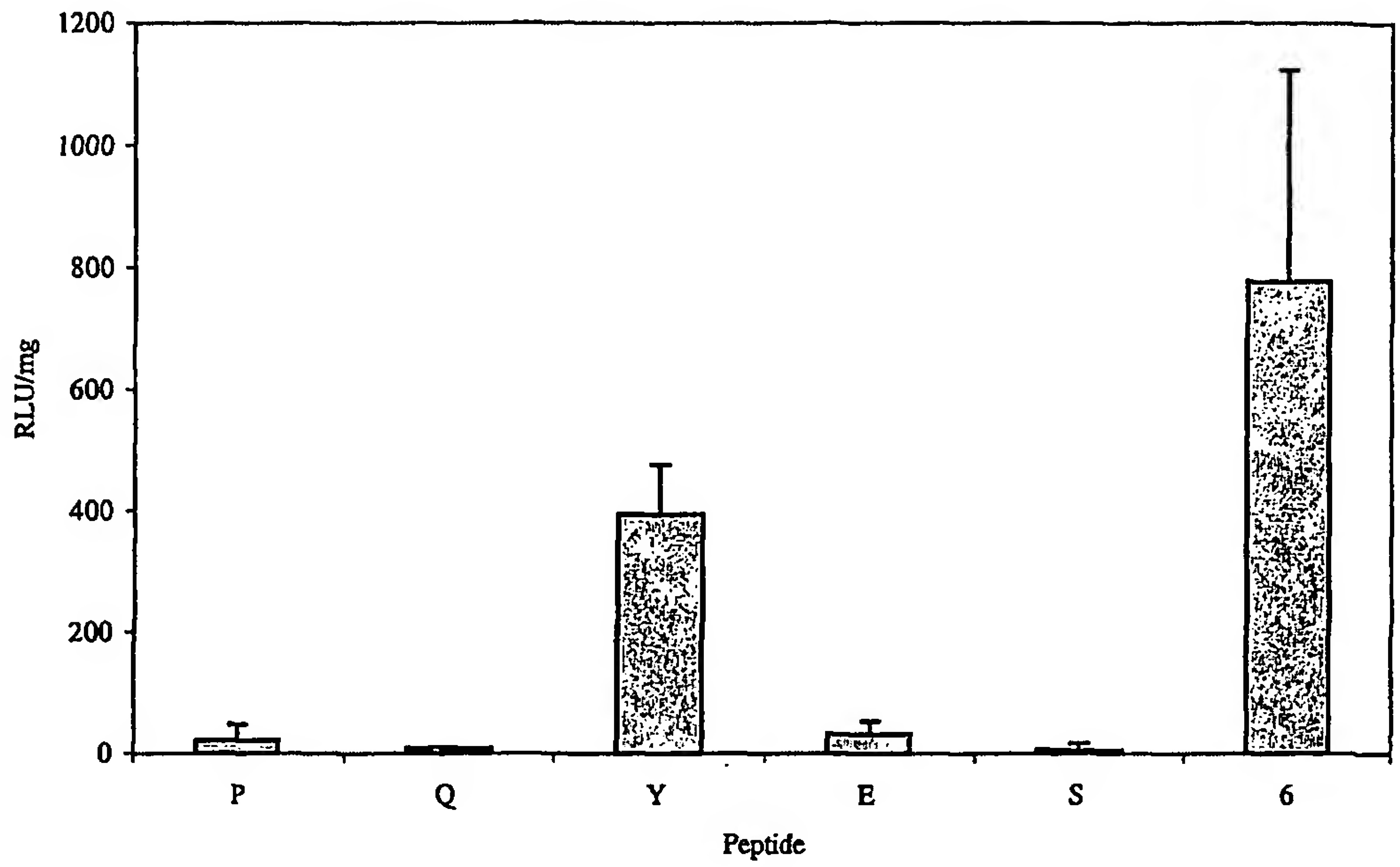

Figure 7

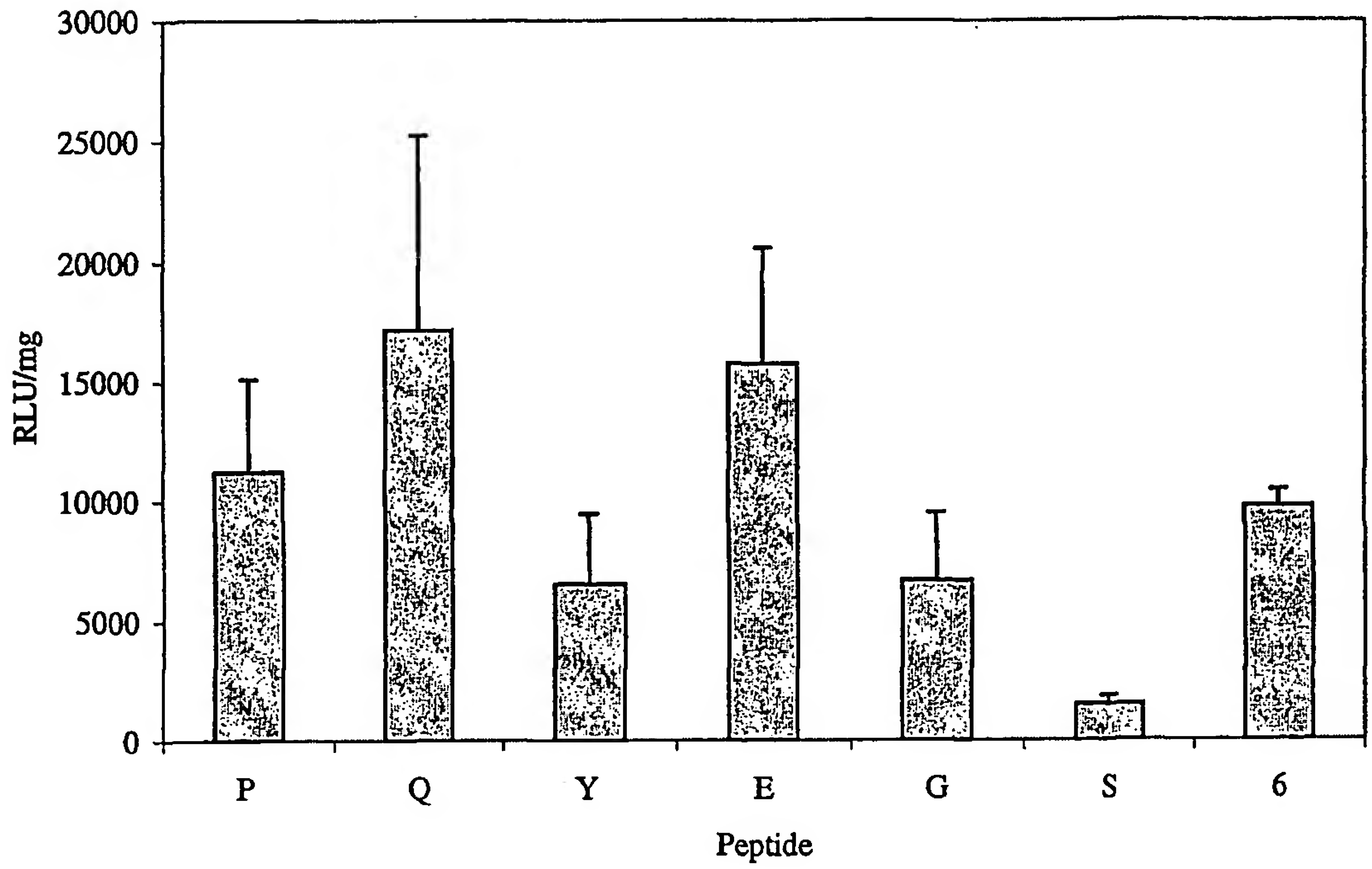

Figure 8

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 September 2002 (19.09.2002)

PCT

(10) International Publication Number
WO 02/072616 A3

(51) International Patent Classification[7]: C07K 5/09, 5/103, 7/06, C12N 15/88, A61K 38/02, 38/06, 38/07, 38/08, 39/00, 48/00, G01N 33/68

(21) International Application Number: PCT/GB02/01215

(22) International Filing Date: 14 March 2002 (14.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0106315.5 14 March 2001 (14.03.2001) GB

(71) Applicant *(for all designated States except US)*: **ICH PRODUCTIONS LIMITED** [GB/GB]; 30 Guildford Street, London WC1N 1EH (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HART, Stephen, Lewis** [GB/GB]; Institute of Child Health, 30 Guildford Street, London WC1N 1EH (GB). **WRITER, Michele** [GB/GB]; Institute of Child Health, 30 Guilford Street, London WC1N 1EH (GB).

(74) Agents: **SILVESTON, Judith** et al.; Abel & Imray, 20 Red Lion Street, London WC1R 4PQ (GB).

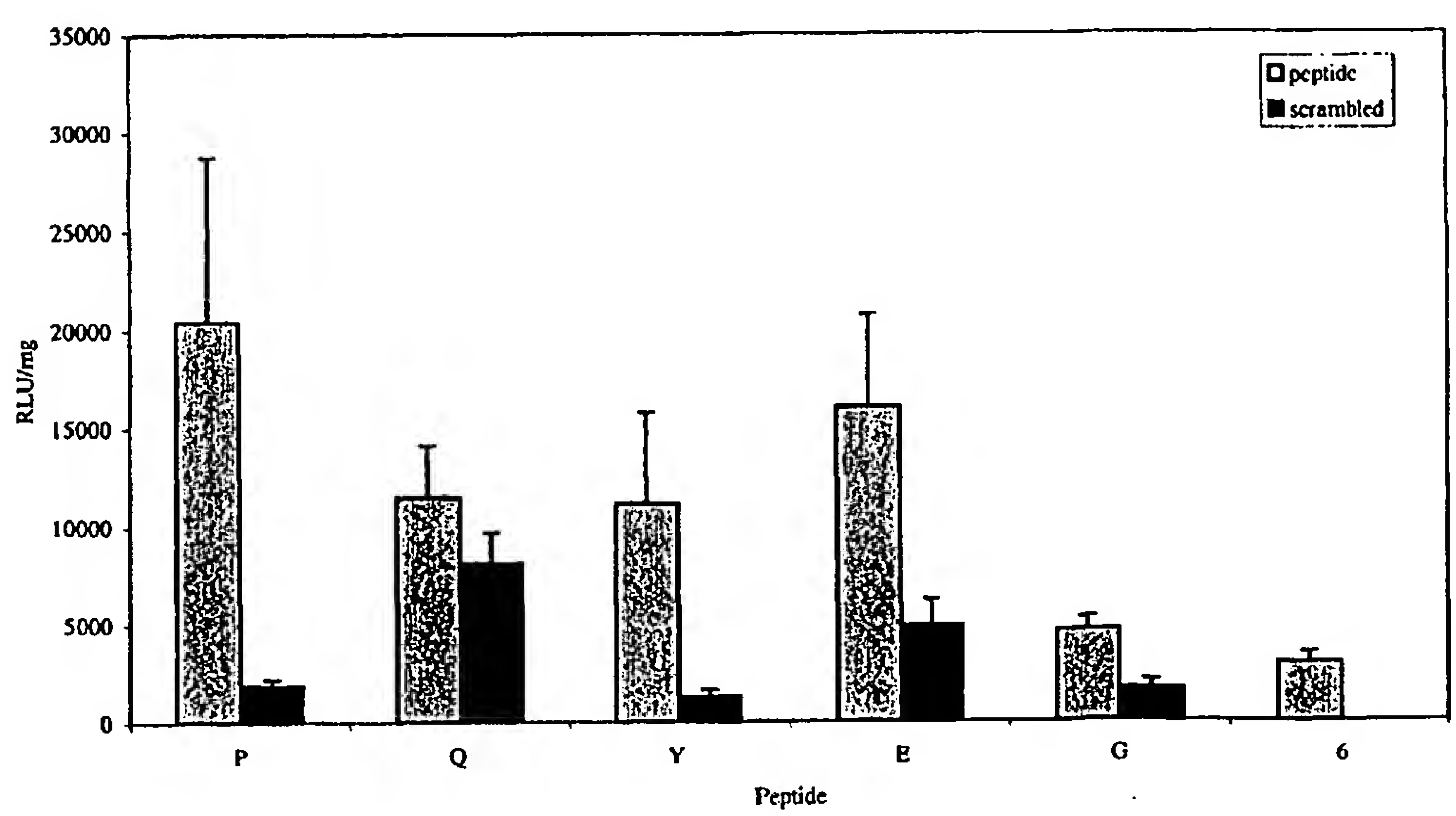
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

[Continued on next page]

(54) Title: TRANSFECTION COMPLEXES



(57) **Abstract:** The invention provides a peptide having at least 3 amino acids comprising an amino acid sequence selected from a) X[1]SM [SEQ.ID.NO.:1] b) LX[2]HK [SEQ.ID.NO.:2] c) PSGX[3]ARA [SEQ.ID.NO.:9] d) SX[4]RSMNF [SEQ.ID.NO.:16] e) LX[5]HKSMP [SEQ.ID.NO.:18] in which X is a basic amino acid residue, X[1] is Q or P, X[2] is A or T, X[3] is an acidic amino acid residue and X[4] is P or Q. the invention further provides non-viral cell-targeting vector complexes and methods associated therewith.

WO 02/072616 A3

(88) Date of publication of the international search report:
3 July 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/01215

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K5/09 C07K5/103 C07K7/06 C12N15/88 A61K38/02 A61K38/06 A61K38/07 A61K38/08 A61K39/00 A61K48/00 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

SEQUENCE SEARCH, CHEM ABS Data, BIOSIS, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EBI 'Online! YDJJ (E. coli), 1 November 1997 (1997-11-01) Database accession no. P77280 XP002226256 abstract ---	1-3,11
X	MAHATA SK ET AL.: "Primary Structure and Function of the Catecholamine Release Inhibitory Peptide Catestatin (Chromogranin A 344-364): Identification of Amino Acid Residues Crucial for Activity" MOLECULAR ENDOCRINOLOGY, vol. 14, no. 10, 2000, pages 1525-1535, XP002226253 figure 2B ---	1-3, 18-20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 April 2003	06. 05. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schmidt, Harald

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HSIEH J-C ET AL.: "Novel Nuclear Localization Signal Between the Two DNA-Binding Zinc Fingers in the Human Vitamin D Receptor" JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 70, 1998, pages 94-109, XP002226254 figure 1	1-3, 18-20
X	KOHLI E ET AL.: "Epitope Mapping of the Major Inner Capsid Protein of Group A Rotavirus Using Peptide Synthesis" VIROLOGY, vol. 194, 1993, pages 110-116, XP002042517 table 1	1-3, 18-20
X	WO 00 75174 A (THE BURNHAM INSTITUTE) 14 December 2000 (2000-12-14) SEQ ID NO 7; page 42, line 8 -page 46, line 24; claims 20-34	1-3, 18-22
X	FEUILLET C & KELLER B: "High gene density is conserved at syntenic loci of small and large grass genomes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, vol. 96, July 1999 (1999-07), pages 8265-8270, XP002237903 abstract & DATABASE NCBI 'Online! Hordeum vulgare, Database accession no. AF108009 abstract	1,2,5, 17,21,22
X	HENDRIX RW ET AL.: "Evolutionary relationships among diverse bacteriophages and prophages: All the world's a phage" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, vol. 96, March 1999 (1999-03), pages 2192-2197, XP000971346 abstract & DATABASE NCBI 'Online! Bacteriophage phi-C31 complete genome, Database accession no. AJ006589 abstract	1,2,5, 15,21,22
X	VELUCCHI M ET AL.: "Molecular Requirements of Peptide Structures Binding to the Lipidd-A Region of Bacterial Endotoxins" VACCINES , vol. 94, 1994, pages 141-146, XP002900364 the whole document	1,2,4, 18,19

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/01215

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 25814 A (UNIVERSITY COLLEGE LONDON) 11 May 2000 (2000-05-11) page 2 -page 5; claims 6,21	1,2,4, 18-22,81
X	SCHNEIDER H ET AL.: "A novel peptide, PLAEIDGIELTY, for the targeting of alpha-9-beta-1-integrins" FEBS LETTERS, vol. 429, 1998, pages 269-273, XP002226255 abstract	81
A	WO 98 54347 A (INSTITUTE OF CHILD HEALTH) 3 December 1998 (1998-12-03) cited in the application the whole document	
A	WO 00 62815 A (GLAXO GROUP LIMITED) 26 October 2000 (2000-10-26) the whole document	

International application No.
PCT/GB 02/01215

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 72,74-76 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-80 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 3,6-12,16,81 (completely) and 1,2,18-80 (partially)

   peptides comprising or consisting of SEQ ID NOs 1,4-7,16,18 and 36, complexes and kits thereof, their processes and methods and a method for identifying a cell surface receptor binding ligand

2. Claims: 4,13,14 (completely) and 1,2,18-80 (partially)

   peptides comprising or consisting of SEQ ID NOs 2,8 and 19, complexes and kits thereof, their processes and methods

3. Claims: 5,15,17 (completely) and 1,2,18-80 (partially)

   peptides comprising or consisting of SEQ ID NOs 3 and 9, complexes and kits thereof, their processes and methods

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-80 (all partially)

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty, e.g. 14764 documents for peptides of claim 3, 2609 documents for peptides of claim 4, 92 documents for peptides of claim 19. So many documents were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claims is impossible. Consequently, the search has been restricted to peptides consisting of or comprising SEQ ID NOs 16 and 18, to heptameric peptides comprising SEQ ID NO 1, to peptides comprising or consisting of SEQ ID NOs 8 and 19, and to peptides consisting of or comprising SEQ ID NO 9 and their complexes, processes, methods and use.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

ormation on patent family members

Inte onal Application No
PCT/GB 02/01215

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